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NOTES AND EXPERIMENTS ON *SARCOCYSTIS TENELLA* RAILLIET

III. IS *SARCOCYSTIS TENELLA* AN ABERRANT FORM OF ONE OF THE CNIDOSPORIDIA OF INSECTS? *

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Several years ago in the first of this series of papers the writer published some results of certain experiments which seemed to favor Darling's insect theory in regard to infection of herbivorous animals with sarcosporidia. More recently it was shown that under Wyoming conditions *Sarcocystitis tenella* is subject to seasonal infection. Notwithstanding this fact the evidence adduced in this second paper was neutral so far as the insect theory was concerned. In the present paper a report will be made of a series of experiments which were intended to show by direct means that insects were responsible for infection with *Sarcocystis tenella*. There will follow a brief discussion of the significance of these experiments. It may be stated here that the results of the experiments were negative, or rather that infection took place independent of all conditions involving insects as the original hosts of the parasite.

The outcome of certain experiments described in the first of these papers led to a favorable opinion of Darling's theory that sarcosporidia of herbivorous animals are aberrant forms of Cnidosporidia that are normally parasitic in the intestines of insects. Accordingly, a series of experiments was arranged in 1915 with the following general plan. It was thought that if the infection was due to a parasite harbored in the intestine of native insects transmission to sheep could possibly take place by two or three methods. A lamb might become infected by eating insects, by eating grass or flowers contaminated with the excreta of insects, or possibly by drinking water in which the parasites had been set free through the death of insects. If the hypothesis proved correct the second method was probably the natural method of transmission, for eating an insect would be rare and accidental, and it would seem the accidental character of the third method would not be likely to account for the almost universal infection of our range

* Experiments by aid of the Adams Fund, Laboratory of Zoology and Parasitology, University of Wyoming.

sheep. The first method, moreover, would be in principle essentially the same as the second. Consequently, all lambs with their ewes, were kept in a dry lot until they were separated into three groups on June 25. Group I, consisting of ten lambs, was allowed to graze daily from June 26 to September 18 in a pasture (1) containing a permanent pond and considerable swampy ground. Previous work had shown that a large percentage of lambs allowed to run in this pasture became infected. Group II, consisting of eight lambs, were allowed to graze in a dry pasture from June 26 to September 18. This pasture contained no water and no swampy ground. Both groups, before and after dates mentioned, were kept in a dry, bare lot, fed baled native hay, a little grain, and given city water derived from deep springs. Group II received city water all summer. Group III, consisting of nineteen lambs, was kept in a dry bare lot all summer, fed baled native hay, given some dry grain, and furnished city water to drink. The details of their further treatment are shown in Table 1. In the first column is given the number of the lamb used in each experiment. In the second column is noted briefly the feeding or other special treatment that each received; tho not given in the table, some of these lambs received an additional treatment in reference to another experiment that dealt with tapeworm infection. This second procedure was unfortunate, as results showed, and tended to involve the problem, but it was thought that if *S. tenella* was a parasite due to insects, it is logical to conclude that the secondary treatment given could have no influence on infection. Accordingly, we shall give a brief explanation and discussion of the results first in relation to the insect theory and then in relation to the secondary treatment, the nature of which is mentioned below.

From the table it will be observed that insects were fed to lambs 203, 204, 205, 214, 216, 217, 221, 256, 257 and 258; that lambs 211 and 219 drank from aquaria contaminated with insects; that lamb 209 was fed flowers from the pasture where the lambs of Group I were allowed to graze; that lamb 210 was fed grass from the same pasture. Perhaps lambs 222 and 224 should be included in this list, since pen 8, from which they were fed grass, is located in pasture 1. Search was made for insects in this pasture twice each week during the summer. A short, cold, wet season contributed to the scarcity of most groups of insects, and only certain flies and mosquitoes were abundant. So far as the insect theory is concerned, lambs 213, 225 and 227 may be regarded as control. The fourth column of the table gives the number of feedings, twenty-two being the maximum, for the summer. Finally, in column five the absence of infection, or if present the apparent degree or amount, is designated. The relative amount of

TABLE 1.—Outline of Experiments in 1915

Lamb No.	Feeding or Treatment	Number Fed	Number of Feedings	Infection
203	Flies	862	22	Mild
204	Moths, butterflies	85	16	Very light
205	Moth larvae	2	2	Light
258	Bees, wasps	32	8	Very light
256	Mosquitoes	1,098	22	None
257	Ants	22	9	None
	Beetlees	19	10	
	Dragonflies	28	9	
	Grasshoppers	9	7	
	Hemiptera	59	9	
	Spiders	33	14	
209	Flowers, pasture I	....	22	Mild
210	Grass, pasture I	....	22	Mild
211	Water, aquarium V, containing killed insects	....	9	None
214	Nosema apis (in 5 dead bees)	Very large	1	None
216	Nosema apis (in 5 dead bees)	Very large	1	Very light
217	Nosema apis (in 5 dead bees)	Very large	1	Mild
221	Nosema apis (in 3 dead bees)	Very large	1	Heavy
219	Water, aquarium IV (Nosema opis in 4 bees)	....	9	None
213	Water, aquarium II (heart muscle containing sarcocysts)	....	11	None
222	Grass, pen 8; feces and proglottids	....	10	Very light
224	Same as 222	....	9	None
225	Grass and 6 insects; from small screen cage; sod contaminated with feces and proglottids	....	3	None
227	None	....	..	None

infection expressed where infection is present should be regarded as of comparatively little value, as this is not based upon numerical data.

In order to account for the wide distribution of sarcosporidia in herbivorous animals, Darling (1915) proposed the hypothesis that herbivorous sarcosporidia were merely aberrant cnidosporidia of insects, and suggested that the habit of depositing excreta on flowers and grass by certain insects, such as bees, moths, etc., would probably be found a sufficient explanation to account for infection. If this were true, feeding the insects ought also to produce the infection. This was the basis of the series of experiments in 1915. A modification of this plan is shown in the case of lambs 211 and 219, where the lambs were made to drink from aquaria into which dead insects were thrown, and in the case of lambs 209 and 210, which were fed very considerable quantities of flowers and grass that had been exposed to insects in pasture 1. This last method would conform to normal infection according to Darling's hypothesis. A close study of the results gave no definite indication that insects either were or were not the cause of infection. For infection apparently resulted from feeding 862 flies, from feeding 85 moths and butterflies, from feeding 2 moth larvae, from feeding 32 bees and wasps, from feeding to each of three lambs 3 to 5 bees that had died with the Isle of Wight disease which is due to the Cnidosporidian, *Nosema apis,* from feeding flowers, from feeding grass, but it did not occur as the result of feeding 1,098 mosquitoes, or as the result of feeding various other insects and arachnids, including ants, beetles, bugs, dragon flies, grasshoppers and ground spiders.

infection also failed in one lamb after feeding 5 bees that had died from infection with *Nosema apis*. Both lambs exposed to aquaria containing various killed insects, including bees infected with *N. apis*, remained uninfected. If infection was due to insects it was derived from three widely different orders namely, Diptera, Lepidoptera and Hymenoptera, and it occurred as the result of eating insects or as the result of eating flowers and grass exposed to insects. One might suppose that the infection was derived from spores deposited on the hay by insects before it was baled. But considering the delicate character of the spores, this was not thought probable. Or, one might suppose the infection due to excreta of certain insects (some flies) that visit dry lots as well as pastures. One thing appeared certain: the *infection with S. tenella could not be due to N. apis*. For infection occurred independent of this parasite (lambs 203, 204, 205), and in one case it did not occur in a lamb when the parasite was known to be present.

Any other theory to account for infection, based on the special treatment received by the lambs, appeared equally unsatisfactory. Of sixteen lambs fed grass or water contaminated with feces, by the secondary treatment mentioned, eight were infected and eight not infected. Lambs 203 and 204 which had received tapeworm proglottids were both infected; the tapeworms appeared to be clean, but of course they might carry some fecal contamination. But if the infection occurred thru contamination with feces, one may well ask why were not all the lambs infected? For all lambs of Group III were exposed to the feces of the ewes running with them in the same lot, and we know that nearly 100 per cent. of our range ewes are infected with *S. tenella*. On the whole, the experiments were deemed inconclusive, since we had not been able to control the infection.

Mention should be made of the results in lambs of Groups I and II. In Group I, including the lambs which were allowed to graze in a pasture containing a pond and some swampy ground from June 26 to September 18, six out of ten lambs were found with sarcocysts. In Group II, grazed in like manner in a dry pasture, sarcocysts were found in only three out of eight lambs. These results indicated that moist or swampy pasture conditions favored infection. The significance of these results became more apparent in later work. Not much emphasis should be placed on these results, for stained sections were not prepared, and subsequent work has shown that some parasites (and probably some infections) will be overlooked unless this is done.

The unsatisfactory outcome of the year 1915 led to a new series of experiments in 1916. The failure to gain control over infection emphasized the importance of carrying out the details of the experiments in a very careful, rigid manner. Again the lambs with their

accompanying ewes were divided into three groups, but the groups received somewhat different treatment. Group I, consisting of six lambs and several ewes, was allowed to graze during the summer in pasture 1. The pasture, however, had been altered in the following way: the pond previously mentioned together with a grassy slope above it was fenced off, and this fenced-off portion will hereafter be called lot III. The lambs of Group I therefore had daily access during the summer to a pasture containing considerable wet and swampy ground. Group II consisted of 22 lambs which were kept in a dry, bare lot from the time they were born until they were killed; they were given dry grain feed, watered in troughs with city water, and up until about September 15 were fed native hay kept over from the previous season; after this date these lambs were fed hay cut during the summer of 1916, since the old hay could no longer be obtained. The ewes to which these lambs belonged were kept with them in the dry lot. The individual treatment of this group of lambs is shown in Table 2, and will be mentioned later; Group III, consisting of six lambs, numbered consecutively 261 to 266, were dept in a dry, bare lot and pastured twice per week in lot III mentioned above. This lot was about 70 feet wide by 100 feet long; the pond and its borders covered most of the lower third on one side; the remainder was a dry grassy slope from 2 to 7 feet above the pond. No ewes or lambs had been on this lot since the previous season, and no ewes were allowed in lot III during 1916. So the lambs of Group III had contact with ewes only while they were in the dry lot.

TABLE 2.—Infection in 1916

Lamb No.	Feeding or Treatment	Number Fed	Number of Feedings	Infection
72	Grass from pasture I	....	15	Very light
73	Flowers from pasture I	....	15	Rather light
74	Flies	1,183	15	Light
75	Mosquitoes	137	7	Medium
76	Moths	65	13	None
77	Butterflies	10	5	Very light
78	Lepidoptera larvae	68	10	None
79	Bees	7	4	Light
80	Wasps	27	8	Very light
82	Ants	12	3	Light
	Beetles	482	12	
	Bugs	44	8	
	Dragonflies	37	13	
	Grasshoppers	54	14	
	Spiders	67	10	
81	Control	....	....	Very light
86	Control	....	....	None
	Control	....	....	Very light
85	Control	....	....	Very light
268	Control	....	....	Light
269	Control	....	....	Light
270	Control	....	....	None
271	Control	....	....	Light
272	Control	....	....	Light
267	Water from pond in lot III	....	12	Very light
273	Grass in pen, lot III	....	13	Medium
275	Grass in pen, lot III	....	13	Very light

Only lambs of group II are shown. See text for other groups.

An inspection of Table 2 shows a plan quite similar to that of 1915. Lamb 72 received grass and lamb 73 received flowers on fifteen different days between July 18 and September 23. At each feeding, which was done individually and by hand, the parts of flowering plants obtainable in pasture 1 were selected with the idea that they had been contaminated with the excreta of insects. With a similar idea numerous tufts of grass from different parts of the pasture were collected for each feeding of lamb 72. During the season one lamb was fed 1,183 flies; another 137 mosquitoes; another 65 moths; another 10 butterflies; another 68 lepidoptera larvae; another 7 bees; another 27 wasps, and still another was fed a miscellaneous group of insects including 12 ants, 482 beetles, 44 bugs, 37 dragonflies, 54 grasshoppers and 38 spiders. Nine lambs, numbers 81, 84, 85, 86, 268, 269, 270, 271, 272, received no special treatment, and were kept as a control. On twelve days lamb 267 was given water from the pond in lot III. On thirteen different days between July 17 and September 11 two lambs, numbered 273 and 275, were grazed in a small pen located in lot III. This pen had formerly been used as a feed pen for small pigs, but had not been used for several years; it was 10 by 12 feet in size, overgrown with grass, and no ewes or lambs had been inside of it for at least two years; it was located on the dry grassy slope of lot III some distance above the pond.

The lambs were all killed during the following winter at various times between November 3, 1916, and March 16, 1917. Material was preserved, sectioned, stained and carefully examined for parasites. The last column of Table 2 gives the general results. Group I, consisting of six lambs that grazed all summer in pasture 1, showed 100 per cent. infection. Group III likewise showed 100 per cent. infection; these lambs were grazed on fifteen different days, between July 17 and September 11, in lot III which surrounded the small pond. Of the two lambs grazed in the small pen in lot III, both were infected. So also were lambs 72, fed grass, and 73, fed flowers. Lamb 267, watered twelve times from the pond in lot III, showed a light infection. Of the nine lambs composing the control seven were infected, and the parasite was found in six out of eight of the lambs which were fed insects. Infection therefore took place independent of the control, and feeding insects did not have any discernible effect either in the number of lambs infected or in increasing the heaviness or degree of infection. A comparison with the lambs fed insects the previous year furnishes some further evidence that insects were not related to infection. For example, lamb 205 (1915) had a light infection after feeding only two moth larvae, while no infection was found in lamb 78 (1916), altho it had received 68 lepidopterous larvae, mostly those of moths. Again, no infection resulted after the feeding of 1,098 mos-

quitoes to lamb 256 in 1915, while feeding 137 of the same insects was followed by a medium infection in lamb 75 in 1916. The feeding of a miscellaneous group of insects and arachnids apparently produced no infection in lamb 257, and the same treatment given to another lamb the next year was followed apparently by a light infection.

From these results it was evident that infection occurred independent of the experiments that were planned to show the direct connection of insects with *S. tenella*. So if insects were responsible for infection, the parasite must be limited to such insects as were common to all of the general conditions present. Now the insects commonly present in the dry lots were limited to certain species of flies, principally houseflies and stable flies, and these were also present in the pastures and lots used. But such evidence as we had was rather opposed to the idea that these insects were responsible for the infection. For 862 flies were fed to lamb 203, and 1,183 flies were fed to lamb 74, and neither of these lambs showed more than a moderate degree of infection; while the number of flies fed included several different species, houseflies and stableflies formed a considerable part of the total number. It was seen, therefore, that we had found no evidence favoring the insect hypothesis. But since the character of the evidence was largely negative there still remained the possibility that the theory might be true.

Accordingly, in the next series of experiments it was planned to arrange the details in such a way that if infection occurred it would exclude the possibility of infection by insects. At the same time some insect and flower feeding experiments were continued. Table 3 shows the general details of these experiments. Mosquitoes were fed twice weekly to lamb 282. Flies were fed to lamb 286; bees to lamb 288; flowers to lamb 289. Flowers were also fed to lamb 287, but in this case the flowers were first heated, with the idea of killing any organisms that might be present, and then exposed to insects for several hours in a closed jar; several species of insects were included, but the common flies caught about the lots formed a large part of the number. By this means the grass was well contaminated with the feces of insects. Lamb 286 unfortunately died from an undetermined cause on July 28, after only four feedings of flies; no sarcocysts were found. The other lambs mentioned were fed twice a week as long as insects or flowers were available. Three other lambs a few days after they were born and before insects appeared, were placed in a screen cage where they were kept until long after all insects had disappeared. Of these lambs, No. 300 was fed flies twice a week, beginning July 17 and ending September 29; altogether, it was fed 1,797 flies which for the most part were caught around the dry lots. Lambs 285 and 287 were kept in the screened cage as controls and with no

other treatment. Two ewes, 87, the mother of lamb 287, and 176, the mother of lambs 285 and 300, were also kept in the screened cage. During the summer a small number of houseflies gained access to the inside of the screen cage, but as these were killed as soon as discovered it is believed they did not have any effect on the experiment.

TABLE 3.—TREATMENT AND INFECTION IN 1917

Lamb No.	Feeding or Treatment	Number Fed	Number of Feedings	Infection
282	Mosquitoes	1,809	16	Medium
286	Flies	82	4	None, died July 28
288	Bees	384	16	Light
289	Flowers	....	17	Light
297	Flowers sterilized; exposed to insects	....	17	Light
300	Kept in screen cage; fed flies	1,787	20	Light
285	Kept in screen cage; control	....	....	Light
287	Kept in screen cage; control	....	....	Heavy

Lambs 282, 286, 288, 289 and 297 were kept in a dry lot all summer.

When the lambs were killed it was found that all were infected with *S. tenella* except lamb 286 which died early. Here again it was evident that we had been unable to control infection. The two control lambs, 285 and 287, were both infected, and in these cases infection had clearly occurred without the aid of insects. In fact, lamb 287 had a heavier infection than any other lamb of the season. Again, as in previous seasons, the feeding of insects produced no noticeable effect on infection. Assuming the insect theory to be correct and that *S. tenella* is an aberrant form, one would expect that if any lambs in the screen cage were infected this would be true only of lamb 300; but such was not the case. On this theory one would expect lamb 297 to show a heavy instead of a light infection, for the sterilized plants upon a number of occasions were very generously contaminated with the feces of insects.

From the results of these three series of experiments it was believed that *Sarcocystis tenella* could not be considered an aberrant form of the Cnidosporidia, for infection apparently occurred without the presence of insects. Consequently, Darling's hypothesis on which we had been working was probably no longer tenable. However, considering the small number of lambs raised in the screened cage, it was thought desirable to obtain additional proof. So in the following year four lambs and their ewes were put in a screened cage and kept there until the lambs were killed.

Lamb 1 was born March 14 and put in the cage the following day; lamb 2 was born May 1 and put in the cage May 7; lamb 3 was born May 4 and put in the cage ten days later on May 14; lamb 6 was born May 10 and put in the cage next day. When killed, lambs 1 and 3 showed a medium degree of infection, while lambs 2 and 6 had fewer sarcocysts present.

These lambs were all put in the cage before insects appeared. From time to time one or two houseflies found a way into this cage, but as a daily search was made for such accidental visitors and they were killed as soon as found, it was believed in view of previous results that they did not have any influence on infection with *S. tenella*. On a few occasions, and for a short time only in the early summer, small gnats were observed in the cage; these had been small enough to pass thru the fine meshes of the screen. The ephemeral character of these insects would indicate that they did not harbor Cnidosporidia that would develop into *S. tenella* if transferred to the sheep. But in spite of all precautions to prevent a possible infection by insects, all four lambs were found infected when killed. Of the seven lambs that had been raised in screened cages all, or 100 per cent., were infected; this had not been true of lambs raised in dry lots outside. The percentage of infection had been increased by raising lambs in a screened cage, even tho all possible precautions were taken to protect these lambs from insects. Except for the slight possibility of infection by means of the accidental insects mentioned above, there seemed to be no longer any ground for holding to Darling's theory. There remained only one more experiment to make the proof complete. That was to raise infected lambs entirely independent of insects.

Lamb 97, born January 23, and lamb 98, born January 24, were placed in a screened cage on April 5. There were no flies in the cage at any time before the lambs were killed on July 1, and except for some small gnats and a few small mosquitoes during the latter half of June, no insects were present. As a rule, on the Laramie Plains practically no insects are present before the first of June, and they are seldom common until after the middle of June. Since it is well known that *S. tenella* requires at least four weeks to appear in the muscles after infection, it is clear that such insects as were present could have had absolutely no influence on infection. Nevertheless, lamb 98 was found lightly infected, and one of the sarcocysts was not less than two weeks old. Hence, one is forced to the conclusion that *Sarcocystis tenella* is not an aberrant form of the Cnido-sporidia of insects.

While one can now safely reject the idea that *S. tenella* is an aberrant Cnidosporidian there are still left other important questions to be answered. Does the life history of *S. tenella* require an intermediate host? By what means do lambs become infected? What is the probable life history of this parasite? These and other questions will be discussed in the next paper, after another series of experiments has been described. It is, however, desirable to mention the relation of the results found in this paper to seasonal infection. It is now apparent that the seasonal infection described in the second of this series of papers does not depend upon insects, and the conditions in some of

the experiments strongly favor the idea that no intermediate host is necessary. If this is true the hypothesis that there is an infective stage in the feces of the sheep, similar to that demonstrated for *S. muris* by Nègre and Crawley, acquires added importance, and the delicate nature of the spores as noted by Fantham and Porter, coupled with adverse climatic conditions, is probably sufficient to account for seasonal infection.

It may be mentioned that lambs 97 and 98 furnished a little additional evidence on seasonal infection. Altho more than 22 weeks old when killed July 1, no sacrocysts were found in lamb 97, and the size of the sarcocysts in lamb 98 indicated that infection occurred not earlier than seven or eight weeks previous to this date. While warm periods lasting a week or two may come as early as May or the latter part of April, freezing at night and snows are common up to the first of June. Considering that these lambs were kept under conditions that always had resulted in infection in 100 per cent. of the lambs used, and remembering that infection is rather common in late spring lambs after they are 10 to 14 weeks old, it appears that the limitation of infection in lambs 97 and 98 was due to seasonal influences.

I am indebted to my assistant, Mr. E. C. O'Roke, for a considerable part of the routine work involved in the experiments mentioned in this paper. I am indebted to the Bureau of Entomology, Department of Agriculture, Washington, D. C., for the bees infected with *Nosema apis*.

SUMMARY

1. *Sarcocystis tenella* is apparently not an aberrant form of one of the Cnidosporidia of insects, for lambs become infected with this parasite without insects being present. Darling's hypothesis is therefore probably untenable.

2. It has been found that lambs are more certain of becoming infected, and that the number of parasites per unit of muscle is greater if they are kept closely confined in a screened cage than if they are allowed to run free in an open dry lot.

3. A second host other than the sheep does not seem necessary for the development of *S. tenella*, and this being true, a sexual stage of this parasite will no doubt be found in the intestine of the sheep. The method of transmission and life history will be taken up in the next paper.

NOTES ON THE LIFE CYCLE OF TWO SPECIES OF ACANTHOCEPHALA FROM FRESHWATER FISHES *

H. J. Van Cleave

The complete life cycle is not known for a single typically North American species of Acanthocephala. Species which have served as the basis of work by investigators on other continents have invariably revealed a complicated cycle of development involving at least one other host in addition to the definitive host which shelters the mature worms. Frequently intermediate hosts have been found intercalated between the primary and the definitive hosts. In most species of Acanthocephala there is not an absolutely fixed specificity of hosts, either primary or definitive. Consequently it is probable that species for which the life cycle has been determined in another country may have acquired entirely different species of hosts on this continent. For example, *Gigantorhynchus hirudinaceus* (Pallas), the common acanthocephalon of the hog, has an extremely broad geographical distribution, but it must utilize various primary hosts within the limits of its range for the geographical distribution of the insect larvae which serve as primary hosts in any given locality does not coincide with the geographical distribution of the parasite. Thus Stiles (1892) found that the species of insect larvae which act as primary hosts for this parasite on this continent are entirely different from the ones that are utilized in its European habitat. For similar reasons one could not be justified in assuming that representatives of the species *Echinorhynchus gadi* Müller from fishes of our Atlantic coast undergo a development identical in detail with representatives of the same species from the European continent.

The works of Linton are the only published records which include information concerning larvae of Acanthocephala peculiar to North America. His references are based chiefly upon incidental observations of larvae found in marine fishes. His frequent mention of *Echinorhynchus incrassatus* from the viscera of various species of marine fishes (1891, 1901, 1905) unquestionably refers to the larvae of an undetermined species of Corynosoma as evidenced by his own drawings. He also recorded the occurrence of *"Echinorhynchus proteus"* (1901 : 481) in the mesentery of a flounder. This last may without doubt be referred to the genus Pomphorhynchus, though the specific identity with the European *P. laevis* (= *"proteus"*) is to be

*Contributions from the Zoological Laboratory of the University of Illinois, No. 150.

doubted. Thus two of the larval parasites mentioned in Linton's works stand without any definite relationships to species known to occur as adults in the North American fauna. In addition to the above mentioned records, Linton described (1889) a new species of Acanthocephala, *Echinogaster sagittifer,* from larvae which were encysted in the viscera of marine fishes. At a later date he definitely associated these larval forms with adults which were subsequently discovered. The larvae from which the species was described were in an advanced stage of development, ready to infect the definitive host. Consequently even in this instance of a marine species of Acanthocephala peculiar to the North American continent little is known of the details of its life cycle. Yet this represents as full an account as is to be found in literature, while for the purely freshwater species nothing has been recorded. In no instance has a complete life cycle been outlined and verified by data from experimental infestation of either primary or definitive host.

In the course of extensive laboratory and field investigation upon Acanthocephala, the writer has come into possession of facts relating to the life cycle of two species of these parasite from freshwater fishes. In view of the fact that no data concerning such forms is available in the literature, it seems advisable to publish the results of this investigation in the hope that other investigators may be induced to cooperate in bringing together data bearing upon the development of other species of these parasites. The present paper embodies information relating to certain phases in the life cycle of two species belonging to the genus Echinorhynchus, both of which are apparently peculiar to freshwater fishes of North America.

SOURCES OF DATA

Investigators in specialized fields frequently fail to realize the service they might render to other investigators by making known to them the incidental or accidental information that they may acquire in the pursuit of their special problems. The materials which formed the basis for the present study have in great measure come to the writer through the cooperation of friends who secured the data while carrying on investigations directed along fundamentally different lines. I am under especial obligation to Drs. A. S. Pearse, A. R. Cooper, G. R. La Rue and Director A. F. Shira for specimens and for data concerning the occurrence of larval Acanthocephala. The U. S. Bureau of Fisheries during the summer of 1919 extended to the writer the opportunity of carrying on investigations concerning the life histories of Acanthocephala infesting fishes, but that work has not progressed to a stage where positive data are available from the experiments that were undertaken.

THE LARVA OF *ECHINORHYNCHUS COREGONI* LINKINS

In the course of investigation upon parasites of fishes, Dr. A. R. Cooper encountered heavy infestations of Acanthocephala in the digestive tract of whitefish taken from the Great Lakes region, especially from the Canadian shore of Lake Ontario. The same individuals which bore heavy infestations of these parasites contained numerous amphipods (*Pontoporea hoyi*) in their stomachs. An examination of some of the amphipods revealed the presence of larval Acanthocephala, which, according to unpublished observations by Dr. Cooper, were located in the body cavity. I have examined specimens, both from the body cavities of these amphipods and from the intestine and ceca of the fishes, and have identified them as belonging to the species *Echinorhynchus coregoni* Linkins. The larval forms taken from the bodies of the amphipods were in a late stage of development. In fact, the size and plan of organization of these larvae did not in any manner differ from the juvenile representatives of the same species taken from the intestine and ceca of the definitive host. All of the larvae which I examined had the proboscis fully retracted within the body, so that serial sections were necessary for accurate determination.

Early stages in the development of this species, from the time the embryo leaves the body cavity of the gravid female to the infecting stage, remain unknown. It seems probable that the entire larval existence may be passed in the body of an amphipod which serves as primary host, and that the larva is introduced into its definitive host when some fish, suitable as a definitive host, devours the infected amphipod.

THE LIFE CYCLE OF *ECHINORHYNCHUS THECATUS* LINTON

A number of years ago Director A. F. Shira of the Fairport, Iowa, Biological Station, encountered larval Acanthocephala in amphipods (*Hyalella knickerbockeri*) which he was rearing as food for young small-mouthed black bass at the Homer (Minnesota) station of the U. S. Bureau of Fisheries. The appearance of these larvae in the amphipods was coincident with an outbreak of an epidemic of acanthocephalan infestation among the young bass. Specimens of the parasites, both from the alimentary canal of the fish and from the bodies of the amphipods, were preserved. Through the courtesy of Director Shira, I have been permitted to examine a few of these specimens and have determined that the mature worms from the bass and the larvae from the amphipods both belong to the species *Echinorhynchus thecatus* Linton. The demonstration, in this instance, of the occurrence of larvae of *E. thecatus* in amphipods and the general infestation of the young bass which were being fed these same amphipods under controlled conditions lends strong support in advocacy of the con-

tention that *E. thecatus* may undergo its complete cycle of larval development within the body of an amphipod and reaches maturity when a suitable definitive host devours the amphipod which shelters the infecting stage of the larval parasite.

Data from other sources furnish incontestable evidence that one or more intermediate hosts may be intercalated between the primary and the definitive hosts of *Echinorhynchus thecatus*. Larvae which unmistakably belong to this species have been encountered frequently encysted in the viscera of various fresh water fishes. At the present time no proof of the method by which they come into this host is available. However, it seems probable that they enter in the same manner as found in other species of these parasites. If an amphipod bearing young larvae of *E. thecatus* is eaten by a fish the young larvae would be liberated in the digestive tract of the fish, but being imperfectly developed would not be able to maintain themselves as intestinal parasites of the new host. Such early larvae probably penetrate the wall of the digestive tract of their new host and become encysted in the mesenteries, in the peritoneum, or in the organs lying in the general body cavity. Here they continue development until they reach a stage identical with the fully formed infecting larvae that occurs in the amphipod.

Evidence in support of the above outlined stage in the life cycle of *E. thecatus* has been derived from the study of material collected by Dr. A. S. Pearse who encountered numerous cysts of various size in the peritoneum of the yellow perch. Pieces of the peritoneum containing these cysts were stained and mounted for study. Many of the cysts included numerous hooks (Fig. 2) which were directly recognizable as the proboscis hooks of *E. thecatus*, while others, more fully developed, contained complete larvae (Fig. 3) in the infecting stage. The writer expects at some later date to examine the living cysts and to supplement the present study by an examination of serial sections of these encysted larvae.

The smallest cyst in the material examined which has been definitely recognized as containing a larva of *E. thecatus* is 0.18 mm. in diameter and almost spherical in form (Fig. 2). A definite fibrous wall surrounded this cyst, and others of a similar structure. In the cyst shown in this figure no definite arrangement of the hooks could be observed, though in many of the more advanced stages the inverted proboscis could be definitely recognized in the toto-mounts of the cysts.

In many of the largest specimens, which frequently attained a length of approximately 2 mm., the proboscis of the larva was fully extended as shown in Figure 3. Individuals such as the one shown in this figure represent the end of the developmental processes of the larva, for they have attained the full size and the same organization

of the body as found in the juvenile specimens of the same species removed from the lumen of the intestine of a definitive host (see Fig. 4).

It is significant that fully formed larvae of the infesting stage are found in Hyalella, while much smaller, less fully developed specimens have been observed in a vertebrate which serves as an intermediate host. This would indicate that the intermediate host in this instance serves not only as a repository for larvae which accidentally enter its body, but also furnishes suitable conditions for completion of the development of the immature larvae which has not gone far on its course when its sheltering primary host is eaten by the intermediate host.

Attainment of the adult body form from a juvenile, such as shown in Figure 4, involves practically no change in the proboscis. Simple increase in size of the body proper and development of the reproductive organs from the rudiments present in the larva constitute the chief developmental processes that proceed after the larva has entered the digestive tract of its definitive host. Since many of the characters utilized in the classification of the Acanthocephala are associated with the proboscis, the early development of this organ renders an identification of larvae possible even though the general body form may be grotesquely different from that of the adult.

The writer (1919) has previously discussed the occurrence of larvae of *E. thecatus* in the viscera of fishes from Douglas Lake, Michigan. Larvae of this species from various organs of fishes examined by Dr. G. R. La Rue are identical in structure with the juvenile forms of the same species which were taken from the digestive tract of definitive hosts from the same locality.

Stages in the development of *E. thecatus* earlier than the one shown in Figure 2 have not been discovered. The form of the larva from the time it leaves the body of the gravid female parasite (Fig. 1) until it reaches this comparatively late stage in its cycle remains unknown.

SUMMARY

1. Some phases in the life cycle of *Echinorhynchus coregoni* Linkins and of *E. thecatus* Linton have been made available, chiefly through the cooperation of other investigators.

2. Larvae of *E. coregoni* have been found in *Pontoporea hoyi*.

3. Pontoporea containing these larvae were taken from the stomach of whitefish which carried an infestation of this some parasite in the digestive tract.

4. Larvae of *E. thecatus* have been found in *Hyalella knickerbockeri*. The young bass fed on these amphipods acquired a general infestation of *E. thecatus*.

5. Various fishes harbor encysted larvae of *E. thecatus* in their viscera and in the peritoneum. An intermediate host does not seem essential for the completion of the life cycle of this parasite.

6. In these two species the transformation from fully formed larva to the adult involves conspicuous changes in the body proper, but practically none in the proboscis.

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Explanation of Plate XIV

Stages in the life-cycle of *E. thecatus*

All figures were drawn with the aid of a camera-lucida from permanent, stained toto-mounts in balsam. Scales accompanying Figures 1 and 2 have the value of 0.05 mm., the others have the value of 0.1 mm.

Fig. 1.—Hard shelled embryo from body cavity of gravid female. Embryos are discharged in about this stage. Cleavage has progressed to a considerable extent.

Fig. 2.—Young larval cyst from peritoneum of yellow perch.

Fig. 3.—Late larval stage ready to infect definitive host. Larva embedded in peritoneum of yellow perch.

Fig. 4.—Juvenile form removed from intestinal cavity of yellow perch. This and foregoing figure drawn to the same scale show identity of form in late larva and juvenile from definitive host.

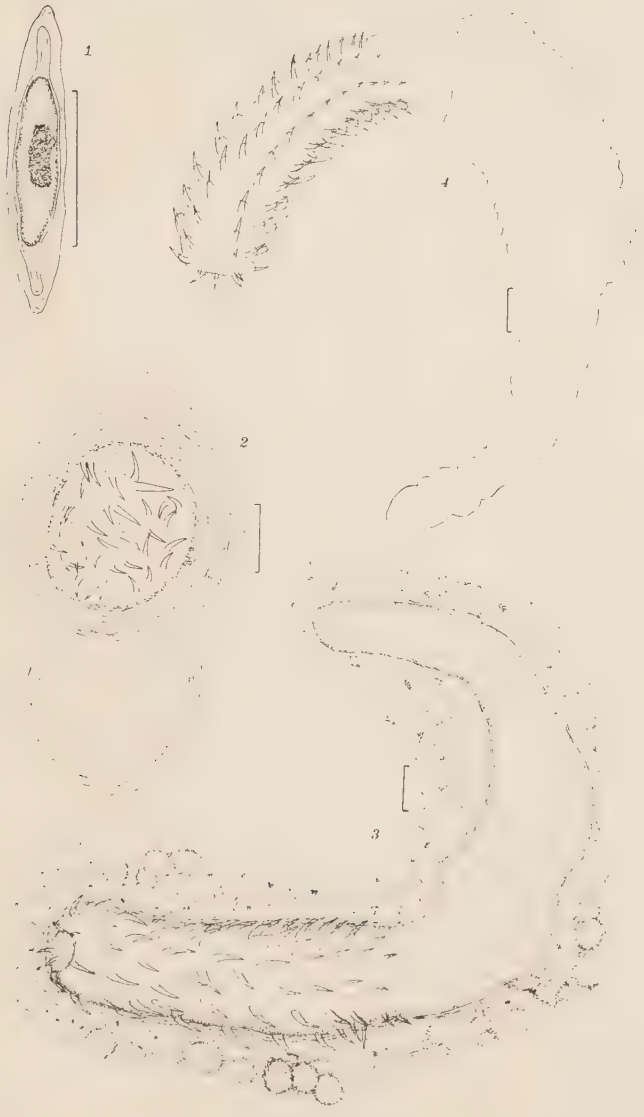

PLATE XIV

SUR LA SOURCE D'INFECTION DU CHIEN ET DU CHAT AVEC *L'ECHINOCHASMUS PERFOLIATUS* (V. RÀTZ) ET LA QUESTION D'INFECTION DE L'HOMME AVEC LES DISTOMES DE LA FAMILLE DES ECHINOSTOMIDÉS

NOTE PRÉLIMINAIRE

PAR J. CIUREA

A ma connaissance la source d'infection de l'homme et des animaux avec les Echinostomes n'est pas encore expérimentalement déterminée; on croyait seulement que cette infection était produite par la consommation de mollusques infestés par des cercaires d'Echinostomes, ou par la boisson des eaux dans lesquelles se trouveraient ces cercaires. Jusqu'à présent on ignorait que les poissons et spécialement ceux d'eau douce pouvaient être considérés comme source d'infection avec ces Distomes.

Dans ce qui suit je veux faire connaitre la source d'infection avec *l'Echinochasmus perfoliatus* v. Ràtz, parasite de l'intestin du chien, du chat et du porc.

Au cours des années 1913-15 nous nourri des chiens et des chats avec différentes espèces de poissons du Danube, dans le but de déterminer lesquels de ces poissons servent comme hôtes intermédiaires aux larves des Opisthorchiidés; et à l'occasion de ces recherches, à l'autopsie des animaux d'experience, nous avons trouvé plusieurs fois dans leurs intestins grêles des exemplaires de *l'Echinochasmus perfoliatus*.

En ce qui concerne les animaux d'expérience (chiens et chats) nous tenons à mentionner qu'ils étaient de jeunes sujets, en grande partie élevés par nous, et qui avant l'expérience n'avaient jamais mangé de poissons, de mollusques ou bu d'eau dans laquelle l'on pouvait soupçonner la présence de mollusques. Pendant nos recherches, ces animaux ont été tenus dans des cages séparées. Comme nourriture, ils recevaient des poissons crus, et quand nous n'en avions pas nous leur donnions du foie de bouf. Cette nourriture leur était distribuée par nous-même.

Les expériences qui font le sujet de notre travail sont les suivantes:

Première série d'expériences avec le *Scardinius erythrophthalmus* et la Brême (*Abramis brama*).

Trois petis chiens de la même nichée, dont l'un a été nourri depuis le 5 Avril jusqu'au 17 Juin (74 jours) avec *Scardinius erythrophthalmus*, un autre chien pendant la même période de temps fut nourri avec 5 grands exemplaires d'*Abramis brama* et le troisème chien nous servit comme contrôle.

Le résultat de l'autopsie des ces trois chiens qui eut lieu le 19 Août (137 jours après le commencement des expériences) fut le suivant: Dans le contenu intestinal du chien nourri avec le *Scardinius erythrophthalmus* nous avons trouvé 5 exemplaires d'*Echinochasmus perfoliatus;* dans celui qui fut nourri avec de la Brême seulement 1 exemplaire; et chez le chien contrôle on ne trouva aucun Trematode dans l'intestin. Le contenu intestinal fut toujours conservé dans 70% alcool et examiné avec la plus grande attention au microscope.

Deuxième série d'expériences. Des chiens et des chats nourris avec la Tanche (*Tinca tinca*), le Brochet (*Esox lucius*), le Carassin (*Carassius carassius*) et l'*Aspius aspius* (l'année 1913).

Deux petits chiens, l'un d'eux a été nourri depuis le 16 Août jusqu'au 3 Novembre avec de la Tanche (*Tinca tinca*). Il a mangé 71 exemplaires de cette espèce de poisson pendant 80 jours. Son frère reste comme controle. A l'autopsie des ces deux chiens sacrifiés le 4 Novembre, j'ai recueilli de l'intestin du chien qui a mangé de la Tanche 1488 exemplaires d'*Echinochasmus perfoliatus.* Chez l'autre chien contrôle on n'a trouvé aucun Distome.

Trois petites chattes soeurs dont l'une a consommé en l'espace de 66 jours (du 1er Septembre au 6 Novembre) 44 Brochets (*Esox lucius*), une autre a ingéré en 60 jours (du 23 Septembre jusqu'au 23 Novembre) 88 Carassins (*Carassius carassius*) et la troisième a servi pour le contrôle.

Après les avoir sacrifiées nous avons recueilli 33 exemplaires d'*Echinochasmus perfoliatus* de la chatte qui fut nourrie du Brochet et 5 exemplaires de celle qui mangea du Carassin. A la chatte contrôle on ne trouva aucun Echinostome.

Deux petits chiens frères dont l'un a été nourri pendant deux mois (depuis le 23 Octobre jusqu'au 23 Décembre) avec 55 exemplaires d'*Aspius aspius,* l'autre servit de contrôle. A l'autopsie du chien nourri d'*Aspius aspius* on trouva dans le contenu intestinal 67 exemplaires d'*Echinochasmus perfoliatus,* chez le chien contrôle aucun exemplaire.

Troisième série d'expériences. Des chiens nourris avec l'Ide jesse (*Idus idus*), Carpe (*Cyprinus carpio*), Barbeau (*Barbus barbus*) et *Blicca björkna* l'année 1913-15.

Quatre chiens dont l'un a mangé depuis le 4 Novembre 1913 jusqu'au 31 Mars 1914 (5 mois) 14 exemplaires de l'Ide jesse (*Idus idus*). A l'autopsie de ce chien on trouva dans l'intestine 540 exemplaires d'*Echinochasmus perfoliatus.* Dans l'intestine du deuxième qui a ingéré pendant plus l'ume anné (27 Décembre 1913 jusqu'au I Février 1915) 65 Carpes et dans l'intestin du troisième chien qui fut nourri dans le même espace de temps avec 19 Barbeaux on n'a trouvé aucun exemplaire d'*Echinochasmus perfoliatus.* Enfin au quatrième chien qui mangea depuis le 17 Juin jusqu'au 23 Juillet (36 jours) 66 *Blicca björkna* on trouva dans l'intestin 3 exemplaires d'*Echinochasmus perfoliatus.* Chez le chien contrôle on n'a rien trouvé.

Le résultat des ces expériencès est que les chiens et les chats ont été infestés par *Echinochasmus perfoliatus* v. Ràtz en consommant les espèces suivantes de poissons du Danube: *Scardinus erythrophthalmus,* la Brême (*Abramis brama*), la Tanche (*Tinca tinca*), le Brochet (*Esox lucius*), l'*Aspius aspius,* l'Ide jesse (*Idus idus*) et *Blicca björkna.*

De même il résulte que les hôtes intermédiaires de prédilection des larves d'*Echinochasmus perfoliatus* sont la Tanche et l'Ide jesse.

Je veux faire remarquer que les poissons voraces, Brochet et *Aspius aspius,* étant toujours mangés en entier par les animaux d'expériences, il est possible que les exemplaires d'*Echinochasmus per-*

foliatus trouvés chez ces animaux proviennent d'autres espèces de poissons qui se seraient éventuellement trouvées dans leurs estomacs.

Après avoir pris connaissance des poissons qui portent les larves d'*Echinochasmus perfoliatus,* j'ai commencé à faire des recherches pour trouver les larves même. J'ai fait l'examen surtout de la Tanche et de l'Ide jesse puisq'il est résulté de mes expériences que ces poissons sont les hôtes de prédilection pour les larves d'*Echinochasmus perfoliatus.*

Par de minutieuses recherches je suis parvenu à trouver de très petites larves d'Echinostomes enchistés dans les écailles et seulement dans le canal de la ligne latérale, un organe qui n'était pas jusqu' à présent connu comme siège des parasites animaux.

Pour le moment je ne peux donner qu'une description sommaire des caractères de ces larves, mes recherches n'étant pas encore complètement terminées. Les larves sont incapsulées dans des chistes ellipsoïdes à double parois, les dimensions d'un tel chiste sont 0.197 mm. sur 0.147 mm. La larve retirée du chiste, un peu contractée mesure 0.197 mm. en longueur et 0.088 mm. en largeur. La surface de son corps présente de très fines épines. La ventouse orale placée à l'extremité antérieure du corps est un peu plus petite que celle ventrale qui se trouve près du milieu du corps. Le disque adoral est réniforme et porte une série de 27 bâtonnets, petits, disposés sur un seul rang ininterrompu sur la face dorsale.

Maintenant vient la question si cette larve d'Echinostome est celle d'*Echinochasmus perfoliatus.* Ce qui s'oppose à cette identification est premièrement le nombre plus grand de bâtonnets du disque adoral de la larve (27) que celui des exemplaires adultes d'Echinochasmus (24) et secondement la disposition de ces bâtonnets qui chez la larve forment une série ininterrompue tandis que chez l'Echinochasmus adulte ils forment une séries interrompue sur la face dorsale du disque adoral.

Si nous considérons que les bâtonnets et les épines des Trematodes sont des formations cuticulaires qui peuvent se réduirent en nombre ou même disparaître pendant le développement de ces animaux, ce n'est pas trop hasarder d'admettre que la larve dont nous avons parlé soit celle d'*Echinochasmus perfoliatus.* Nous avons aussi des exemples: Ainsi Looss a observé chez *Stephanochasmus bicoronatus*—Trématode très proche des Echinostomiidés—que la double couronne de bâtonnets de l'orifice buccale, laquelle chez les exemplaires normaux est interrompue sur la face ventrale, chez d'outres exemplaires cette double couronne est complétée par la présence d'un nombre supplémentaire de bâtonnets au lieu de 31. D'ou cet auteur tire la conclusion très logique que le type primitif de *Stephanochasmus bicoronatus* aurait eu un plus grand nombre de bâtonnets autour de l'orifice buccale, qui se seraient réduits avec le temps, et que les bâtonnets supplémentaires

doivent être considérés comme des retours à l'état primitif. De même Kobayashi chez *Clonorchis sinensis* et moi chez *Opisthorchis felineus* nous avons montrés que les larves de ces Distomes ont des épines disparaissant complètement pendant leur développment jusqu'à l'adulte, ce qui dénote que le type primitif de Clonorchis et Opisthorchis a eu des épines tégumentaires.

Vu ces exemples on peut supposer que le type primitif d'Echinochasmus ait eu un disque adoral avec 27 bâtonnets et que ce nombre serait réduit avec le temps par la disparition de trois bâtonnets de la face dorsale du disque adoral, et que ce sont seulement les larves qui ont conservé le nombre et la disposition de bâtonnets du type primitif d'Echinochasmus.

Il est intéressant de mentionner qu'Ercolani croyait avoir reproduit expérimentalement l'*Echinostomum echinatum* Zeder, parasite de l'intestin, de la poule et quelques oiseaux aquatiques, chez le chien en lui faisant ingéré *Cercaria echinata* (Sieb.) ; Railliet et Henry sont d'avis que ce Echinostome était *Echinochasmus perfoliatus*. Moi, je suis sûr que ce ne sont pas les mollusques, qui ont infesté le chien d'Ercolani avec cet Echinostome, mais que le chien était infesté avant l'expérience par l'ingération de quelques poissons.

On fait encore mention dans la littérature sur la présence chez les poissons marins de larves d'Echinostomes. Ainsi Stossich nous dit avoir trouvé dans la cavité abdominale de *Gobius jazo* l'*Agamodistoma valdeinflatum* (Stossich) qui représenterait d'après lui la larve d'*Echinostoma cesticillus* de l'intestin de quelques poissons de mer. Fiebiger a trouvé une larve semblable à l'*Agamodistoma valdeinflatum* dans des tumeurs de la peau de *Zeus faber*. Mais nous savons qu'actuellement on place les Distomes du type d'*Echinostoma cesticillus* dans le genre *Stephanochasmus* qui forme un groupe à part voisin de la famille des Echinostomiidés.

Il me parait que Yokogawa dans les dernières années à l'occasion de ses recherches sur les larves de *Metagonimus yokogawai* aie trouvé dans les branches d'une Forelle (*Plecoglossus altivelis*) parmi les larves de Metagonimus encore des larves d'Echinostomes, mais qu'il ne les reconnut pas. D'après Yokogawa ces larves seraient plus petites que celles de Metagonimus, elles sont incapsulées dans des chistes elliptiques qui mesurent 0.105-0.119 mm. de longueur et 0.056-0.07 mm. en largeur. La ventouse orale de ces larves est armée de petites épines. Le corps est immobile et avec une structure confuse. De ces larves on n'a pu déterminer chez les animaux d'expériences le ver adulte.

Quoique la description donnée par Yokogawa à ces larves est incomplète, je crois qu'elles représentent des larves d'Echinostomes.

Le fait qu'en Asie orientale l'infection de l'homme avec *Clonorchis sinensis* et *Metagonimus yokogawai* a lieu par la consommation des poissons crus et que chez ces poissons on pourrait trouver des larves d'Echinostomes, m'a suggéré l'idée qu'aussi l'infection de l'homme aux Philippines par *Echinostomum ilocanum* et à Malacca par *Echinostomum malayanum* serait produite par la consommation des poissons crus. En effet Garrison et Leiper ont recueilli ces Echinostomes de l'intestin des indigènes qui sûrement mangent les poissons ainsi que les Japonais à l'état cru.

Cette supposition trouve sans ancun doute encore un point d'appui dans le fait que j'ai pu déterminer expérimentalement que l'infection du chien et du chat avec *Echinochasmus perfoliatus* est produite par la consommation de quelques poissons crus.

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ON THE STRUCTURE OF SOME MICROSPORIDIAN SPORES *

R. Kudo

The structure of the spores of Microsporidia has been variously described by several authors for different species. Even in one and the same species, the observations of many investigators do not seem to agree. This controversy may be attributed partly to the minuteness of the object and partly to the difference in the technic used. On examining the numerous papers on Microsporidia, one would be impressed by the fact that the majority of the authors do not state their observations with positiveness.

A more or less generally accepted conception of the structure of the Microsporidian spore seems to have been given by Mercier (1908) for *Thelohania giardi* (length 5 to 6μ, after Thélohan). Mercier observed that the spore is covered with a bivalve shell, each valve developing from an uninucleated parietal cell, that the spirally coiled polar filament is contained in a polar capsule with a nucleus, that the girdle-shaped sporoplasm with at first two, later four nuclei, surrounds the polar capsule, and that a vacuole is present at each pole of the spore. This view, on the whole, has been confirmed by Schröder (1908), Stempell (1909), Fantham and Porter (1912, 1914), Strickland (1913), Kudo (1916), and others, altho their observations differ in details.

On the other hand, Schuberg (1910) noticed in the spores of *Plistophora longifilis* (macrospore, 12μ long, 6μ wide; microspore, 3μ long, 2μ wide) that the girdle-shaped sporoplasm which is circular in cross-section, contains a single nucleus, that the polar filament is coiled directly under the shell mostly in the posterior portion of the intrasporal space, that the so-called polar capsule does not occur in the Microsporidian spore, and that the nuclei observed by other authors, are none others than the metachromatic granules. The same view has been maintained by Omori (1912), Weissenberg (1911, 1913) and Debaisieux (1913, 1915).

Léger and Hesse (1916) described an interesting type of Microsporidia under the generic name of Mrazekia. The spores are of cylindrical or tubular form, and show an entirely different structure compared with other genera. The polar filament is differentiated into two parts. No polar capsule is mentioned as present, the polar fila-

* Contributions from the Zoological Laboratory of the University of Illinois, No. 151.

ment being coiled directly inside of the shell. Instead of being in form like a girdle, the binucleated sporoplasm is a rounded and more or less well defined body embedded in a clear space at the posterior portion of the spore.

The same authors (1916a) later reported a similar observation made on the structure of the spore of *Plistophora macrospora* (8.5μ long, 4.24μ wide, after Cépède). They mentioned that the polar capsule lies closely to the shell, occupying the greater part of the intrasporal space, that the polar filament is coiled in the capsule without a central axis, that the sporoplasm is a rounded binucleated body embedded in the posterior vacuole of the spore, that the girdle-shaped structure which was thought to be the sporoplasm by numerous authors is none other than the retracted substance composing the polar capsule so that one or two turns of the polar filament were mistaken in optical cross-section as a variable number of nuclei, and that the granule in the posterior vacuole which was designated as a metachromatic granule by some authors, is none other than the nucleus of the true sporoplasm. Georgévitch (1917) agreed with the above mentioned view in his study on *Mariona marionis,* altho he noticed that the polar capsule was entirely absent in some spores.

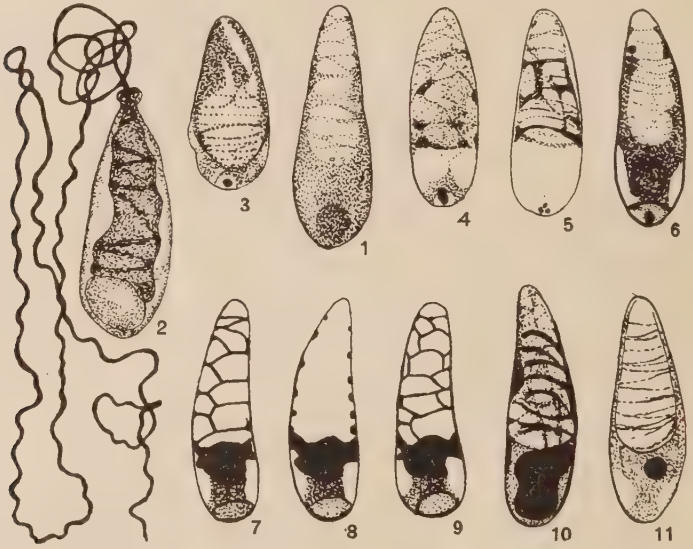
The writer has recently obtained four new forms of Microsporidia from the vicinity of Urbana, Illinois. As their occurrence is rather rare and their life history is now being studied by both natural and artificial infections, it will take some time to complete the work.

One of the parasites, which is a rare parasite in the larvae of *Culex pipiens* from a limited water area in Urbana, proved to be well fitted for the study on the structure of the spore. The spore is from 12 to 13.5μ in length, and 4μ in breadth as measured in stained materials. It is, therefore, one of the largest microsporidian spores that have been recorded. Altho some of the spores of *Plistophora longifilis* and *Thelohania legeri* have the same length, the majority of the spores of these two species are small, while the present form has its advantage in having spores of uniformly large dimensions. Besides, the shell is very thin so that the internal structure could, to some extent, be made out in vivo.

The spore is elongated pyriform usually slightly bent toward one side. It is circular in cross-section. The posterior end is broadly rounded, while the anterior extremity is less rounded, tho not attenuated.

In the fresh state, the spore exhibits marked vacuolation thruout the intrasporal space. For about two-thirds of the anterior portion, a fine polar filament coiled like a network can be seen (Fig. 1), which becomes more distinctly visible when stained, while the posterior one-third is occupied by a finely granulated protoplasmic mass which often

contains a refringent body near its extremity. When fresh spores are subjected to mechanical pressure (Kudo, 1913), and stained by Fontana's method, the extruded polar filament is distinctly recognizable (Fig. 2). This filament is uniformly thick, shows usually a wavy course, and reaches a length of 230μ. The writer does not think this as an average length but records it here as the longest one found so far. In Figure 2 is shown not only the extruded polar filament, but also its remaining part coiled spirally inside of the capsule. The same figure gives at the same time strong evidence for the presence of a particular polar capsule with its polar filament. The shell does not



Spores of *Thelohania magna* nov. spec. × 2360. Fig. 1. A fresh spore. Fig. 2. A spore mechanically pressed, and stained after Fontana. Fig. 3. A young spore stained with Giemsa's stain. Figs. 4-6. Spores stained with Giemsa's stain. Figs. 7-9. Three different views of a single spore stained with Giemsa's stain. Fig. 7. The lower surface view. Fig. 8. The optical section. Fig. 9. The upper surface view. Fig. 10. A spore somewhat deeply stained with Giemsa's stain. Fig. 11. A spore stained with Delafield's hematoxylin.

exhibit any sutural line that might suggest a bivalve nature such as one sees in a Myxosporidian spore either in fresh or stained preparations.

When fixed with Schaudinn's fluid, and stained with Giemsa's stain followed by acetone dehydration, Heidenhain's iron hematoxylin, or Delafield's hematoxylin, the spore shows its various structures very distinctly. Inside of the shell, a large pyriform polar capsule becomes more visible together with the polar filament. The polar capsule, 7.5μ in length, occupies about two-thirds of the anterior portion of the

spore as studied in the fresh state. The foramen of the capsule can not be seen clearly, but the fact that the polar capsule opens at the anterior tip of the spore is distinctly shown in Figure 2. The wall of the polar capsule is comparatively thin, and is very faintly stained in many spores treated with Giemsa's stain (Figs. 4, 5, 7-9). In spores stained deeply with the same stain, however, the polar capsule is recognizable as a reddish colored sack (Fig. 10). In younger spores it is well seen (Fig. 3). It is distinctly recognizable when the spore is brought under the influence of mechanical pressure (Fig. 2). A polar capsule of similar appearance was observed by Schröder (1914) in *Thelohania acuta*, altho the same author did not trace out the filament. The polar filament is coiled spirally along the inner surface of the polar capsule. Its spiral course begins at the anterior tip of the capsule, and does not differentiate a central axis, altho some longitudinal courses were often seen in the posterior portion of the capsule (Fig. 10). Figure 3 shows the developing polar filament in a young spore; the windings are more or less clearly visible. In a deeply stained spore, the spiral can be recognized distinctly (Fig. 10). Three different views of a single spore treated with Giemsa's stain are shown in Figures 7 to 9, which exhibit the spiral course more distinctly along the inner surface of the polar capsule than any other spores. The spirality of the present form is, therefore, somewhat similar to that of *Plistophora macrospora* (Léger and Hesse, 1916a), of *Nosema bombycis* (Kudo, 1916), and of most of the Myxosporidian spores (Auerbach, Kudo, Davis, etc.); but differs from Stempell's (1909) observations on *Nosema bombycis* and from Mrazekia studied by Léger and Hesse (1916).

The rounded sporoplasm occupies the posterior third of the spore. In fixed preparations a clear space is seen on its lateral side (Figs. 6, 7-9, 11). The nucleus is a comparatively large rounded compact mass embedded in the sporoplasm, and shows typical nuclear staining by the above mentioned stains. It is well differentiated in spores stained with Delafield's hematoxylin (Fig. 11). In every spore stained less deeply with Giemsa's stain the nucleus is represented by a single, or two or three smaller chromatic granules situated regularly at the posterior tip of the spore (Figs. 3-6). No nucleus for the polar capsule or the shell has been recognized. Schuberg (1910) noticed a similar fact in *Plistophora longifilis* as was stated before.

The other three forms have spores of much smaller dimensions, and so far have not shown any fact regarding their structure other than the observations which were presented by the present writer in his paper on *Nosema bombycis* (Kudo, 1916).

SUMMARY

The spore of *Thelohania magna* nov. spec. is of exceptionally large dimensions. Microsporidian spores are not so similarly built as those of different genera of Myxosporidia. A diversity in the structure of Microsporidian spores is recognized with at least two categories: one type, *Nosema bombycis* and the other type, *Thelohania magna*. The latter has a distinct polar capsule with spirally coiled polar filament without central axis; it has a rounded sporoplasm containing a single nucleus. Combination of mechanical pressure and Fontana's staining is especially favorable for the study of the extruded polar filament, and also some structures in the spores. To this type may belong *Thelohania acuta, Plistophora elegans* and *P. macrospora*.

It is interesting to note that altho the parasite attacks only the adipose tissue of the host, infected larvae die more rapidly in captivity than normal ones. So far pupae and adults have been found to be free from infection, which suggests a fatal effect of the parasite upon the host body.

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OBSERVATIONS ON ABNORMAL COURSES OF INFECTION OF *PARAGONIMUS RINGERI* *

SADAMU YOKOGAWA AND SUSUMU SUYEMORI

It is a well known fact that infection with *Paragonimus ringeri* is caused by the swallowing of the encysted larvae. It is, however, important to know whether these larvae, if freed from their cysts, can infect through the skin, mucous membrane or any wound on the skin of the host. It seems that this might be possible since they can penetrate the intestinal wall, diaphragm, connective tissues, muscles and some of the viscera of the host after the encysted larvae are swallowed. To test the possibility of such infections, we carried through experiments in an attempt to answer the following questions.

1. Can the freed larvae penetrate the sound skin of the host? The larvae after being freed from their cysts are injured in fresh water, and lose their power of movement, so that some other medium was necessary for the experiments. They are more active in artificial intestinal juice or in normal saline. To determine whether active larvae can penetrate the skin of the host under suitable conditions we experimented on mice, cats and new-born puppies. The results of these experiments are as follows:

(a) Although the freed larvae move actively in the artificial intestinal juice or normal saline, yet they can not penetrate the sound skin of the mice and puppies in a room temperature below 30° C., since their movement decrease below 37° C.

(b) We stretched three mice on a small plate after shaving off the hair of the abdominal wall and dropped upon them the artificial intestinal juice containing the freed larvae. We then put them into a warm chamber at 38° C. After one and a half hours we found that in one case there was a slight desquamation on the abdominal wall, but we could find no evidence of penetration by the larvae.

These experiments prove that the freed larvae cannot penetrate through the sound skin of the host.

2. Can the freed larvae infect through a wound in the body? It is conceivable that the freed larvae might penetrate a wound on the body. It is necessary, however, to try by experiment whether they can actually infect from an exposed wound of the host. On July 15, 1918, we dropped the normal saline with many freed larvae on fresh wounds, which we made in the backs of two dogs. After awhile we found

* From the Pathological Department of the Medical College of Formosa and the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University.

several points of hemorrhages caused by the perforation of these larvae. Then we covered the wound with a watch glass and bound it to the body in order to prevent infection from the licking of the wound. One of the dogs died on August 20, thirty-five days after the experiment, and the other on September 10, having passed fifty-seven days after the experiment. By dissection, we could not find any distomes in the first dog, but in the last case two freed distomes were discovered in the chest cavity.

By these experiments we proved that occasionally the freed larvae can infect a host from a fresh wound on the body.

3. Can the freed larvae infect the host through the mouth? In Korea, R. Kawamura *(Tokyoer med. Woch. No.* 1986, 1916*)* proved experimentally that *Paragonimus ringeri* which is growing in its final host, can continue development after being fed to other animals. This mode of infection is very interesting in relation to lung distome disease, because many Koreans have the habit of eating the meat and the liver of dogs and other animals raw. If such animals had the young distomes wandering in their muscles, connective tissues and liver, this habit might lead to the infection of man. Therefore, we examined this point very carefully, using seven dogs, with these results:

(a) A new-born puppy was fed with 25 larvae, which were just freed from the cysts in the artificial intestinal juice, on July 31, 1917. It was killed on October 2, sixty-one days after feeding. On dissection, we found a wormcyst in the middle lobe of the left lung, in which were two distomes, and a freed distome in the left pleural cavity.

(b) A new-born puppy was fed with 25 larvae which had just been freed from their cysts, on Aug. 2, 1917. The puppy died on August 23, having passed twenty-one days after feeding. On dissection the next day, we found a distome in its right chest cavity, and numerous ankylostomes and ascarids which caused the death of the dog.

(c) A young dog was fed on Dec. 9, 1916, with 15 distomes which had lived for forty-two days in another dog, and killed on the twenty-fifth of the same month, fourteen days after feeding. On dissection we could not find any worms in the body.

(d) A young dog was fed on Aug. 19, 1918, with 20 distomes, which had lived for forty-two days in another dog. The dog died on the thirtieth day of that month, ten days after feeding. On dissection, we could not find any distomes in its body.

(e) A young dog was fed on July 8, 1918, with 27 distomes, which had lived for fourteen days in another dog, and 51 distomes on the thirty-first of the same month, which had lived for twenty days in another dog. Next day we killed this dog, five days having passed after the first and one day after the second feeding. On dissection, an hemorrhage was observed in the intestinal wall. This hemorrhage

probably was caused by the action of the distomes, therefore we looked for the worms with special care, but could not find any worms in the body.

(f) A young dog was fed on June 15, 1918, with 18 distomes, which had lived for eighteen days in another dog, and on the nineteenth of that month with 9 distomes which had lived for twenty-three days in another dog. On June 21 it was killed, having passed six and two days after first and second feeding. Careful dissection failed to disclose any worms.

(g) A young dog was fed on June 10, 1918, with 228 larvae just freed from their cysts and killed twenty hours after feeding. On dissection, some inflammation was observed here and there on the serous membrane of the viscera, and many hemorrhages were found in the intestinal wall. It is evident that these hemorrhages were caused by the perforation of the distomes, because we found 21 distomes in the abdominal cavity. The diaphragm and the lungs were intact. There were no distomes in the pleural cavity.

From these experiments we learned that the larvae which were just freed from the cysts as well as the encysted larvae can infect by way of the mouth, but that distomes which were in a more advanced stage of development in their host, cannot develop after feeding to other animals. This indicates that the worms, which are partly developed in one host, find it difficult to pierce the wall of the intestine when introduced into another host, because of the decrease in activity which comes with growth. We proved this fact by using the mucous membrane of lips and the conjunctiva of dogs. For example, if we put some newly freed larvae on the conjunctiva, or on the mucous membrane of the lips of a dog, they soon penetrate the mucous membrane, but the distomes, which are partly developed in one host, cannot bore through these membranes. While our experiments showed that partly grown larvae of *Paragonimus ringeri* could not be transfered from one host to another, two cases of such infection have been reported by Mr. R. Kawamura and by Dr. Ando* (*Rept. Jap. Path. Soc.* v. 6, 1918). On account of these exceptional cases, it will be necessary to forbid the eating of the raw flesh and livers of animals which can harbor the lung fluke.

4. Can the freed larvae infect through mucous membranes outside the digestive tract? To ascertain the pathological changes in the orbits caused by *Paragonimus ringeri,* we dropped normal saline containing larvae just freed from their cysts, on the conjunctiva of dogs,

* Dr. Ando later repeated this experiment, using eighteen white rats. His results in this second experiment failed to show development in a second host of larvae which had lived for a period in one final host (*Tokyoer Med. Wochenschr.*, No. 2163, 1919).

cats and rabbits. In a little while, we found some small hemorrhages, which were caused by the penetration of the worms. In this experiment, nine rabbits, seven dogs and two monkeys were used, and were examined at various times after these experiments. Each animal showed the presence of the distomes after careful examination first of the orbits and then of the whole body. We proved that the distomes which entered into the orbits were to be found in that place for ten or fifteen days after the beginning of the experiments. But in the cases in which twenty days or more had passed after the experiment, they were found in the chest cavity and not in the orbits. It is very interesting to know how they found their way from the orbits to the chest cavity. We demonstrated this point on experimental animals, and will describe the two most interesting cases.

(a) On September 1, 1918, we put 14 distomes into the right, and 17 into the left orbit of a young dog by cutting open the capsule of Tenon. These distomes were collected from a dog, which was fed with a large number of the encysted larvae sixty-one days before. The dog died on the ninth of that month, eight days after the experiment. On dissection, there was found some muddy exudate in the pleural cavity. The lungs were congested a little and showed some irregular points of hemorrhages here and there, but we could not find any pathological changes, caused by penetration of the worms. We found a distome along the vena cava superior, near the lower end, of the trachea, and another distome on the diaphragm of the right side. In the dissection of the neck we found a distome, which was moving in the loose connective tissue of the right side, about the middle of the trachea, and another distome in the submucosa of the posterior wall of the pharynx. Both eyeballs were badly injured by the operation, but we could not find any worms in the eyeballs and in the orbits. We found only a small suppuration, which was due to the boring of the worms, and the subsequent infection by bacteria, in the tissue of the upper corner of the left orbit and in the left temporal muscle.

(b) On October 11, 1918, we put four distomes into the right and fourteen into the left eye of a big dog by the same operation. These distomes were all mature. The dog died on the twenty-fifth of that month, 14 days after the experiment. On dissection, 2 distomes were found; one of them was situated on the bifurcation of the internal maxillary artery and the superficial temporal artery, and the other worm was located in the masseter muscle around the masseter artery. The lungs and the other viscera were intact.

From these experiments we concluded that the distomes which were dropped on the conjunctiva, penetrate into the orbits and live there a certain number of days. Afterward they escape from the orbits and move to the chest cavity, wandering in the soft tissues.

Therefore, in the monkey's orbits, which are enclosed completely by the bones, they remained a very long time. In the case of a monkey, which was examined eighty-four days after the experiment, we found a living distome in the orbit instead of in the chest cavity.

SUMMARY

1. Young active larvae of *Paragonimus ringeri,* just freed from cysts, cannot penetrate the sound skin, but can enter through a fresh wound.

2. Such young distomes can infect the body by the mouth. Their course from the intestine to the lungs is very similar to the route taken by the encysted larvae.

3. Young distomes just freed from cysts can penetrate the mucous membrane outside of the digestive tract, like the conjunctiva, and bore through the tissues until they reach the lungs.

4. Our experiments indicate that other animals cannot be infected by distomes, which had started development in the final host.

5. Partly developed distomes cannot penetrate the mucous membrane, but, if transferred to the orbits of a suitable host, they penetrate the tissues and reach the lungs.

A NEWLY DISCOVERED PARASITIC NEMATODE

(*Tylenchus mahogani*, n. sp.)

CONNECTED WITH A DISEASE OF THE MAHOGANY TREE

N. A. Cobb

United States Department of Agriculture

Tylenchus mahogani, n. sp. $\frac{3.4}{3.7}$ $\frac{15.}{4.8}$ $\frac{21.}{4.8}$ $\frac{42-83-3}{4.1}$ $\frac{96.3}{2.3}$ 0.56 ... The naked transparent, colorless cuticle is traversed by about one thousand plain transverse striae to the millimeter. The striae are more or less easy of resolution, and, owing to their presence, the plain contour of the body becomes more or less crenate on the tail of the female. Two wings occur opposite each lateral field, consisting of two double lines, beginning near the head and ending near the anus, and occupying a space one-third as wide as the body. There are no longitudinal striations in the cuticle, but the attachment of the somatic muscles gives rise to faint longitudinal markings in the subcuticle. The rounded or subtruncate continuous head presents a mouth-opening of the usual character—not at all, or exceedingly little, depressed. The minute lips are thoroughly amalgamated, and present a refractive, six-ribbed framework as the support of a flattish dome very much like that found in related species, such as *Tylenchus musicola*. The lip-region may be described as being flattish hemispherical, and its framework may appear as if made up of a series of six loop-like elements. The tubular pharynx is entirely typical. The refractive spear, which is about as long as the base of the head is wide, is a rather prominent feature. Its base is faintly trilobed and one-fifth to one-sixth as wide as the base of the head. The main shaft of the spear, comprising the posterior half, is one-half as wide as the bulb, and its junction with the tapering anterior half of the spear is marked by a distinct, but exceedingly fine, encircling ridge. The guiding pieces for the spear, forming the axial part of the lip-region and surrounding the spear, are three-fifths as long as the tapering anterior half of the spear. Posteriorly, the neck is more or less cylindroid; anteriorly, it is conoid. No traces of amphids or eye-spots have been seen. The subspherical median bulb of the esophagus is one-half as wide as the corresponding portion of the neck, and is set off by a constriction in front. The anterior portion of the esophagus tapers more or less to the median bulb, so that at the base of the pharynx the esophagus is two-fifths, at the nerve-ring one-fifth, as wide as the corresponding part of the neck. The lining of the esophagus is a distinct feature. Esophageal glands of the usual tylenchoid character appear to be present. The median bulb contains a sub-spheroidal valve one-fourth as wide as

itself. The intestine, which, as is often the case in Tylenchus, begins in a rather indefinite way, has a faint lumen and thick walls, and in cross-section presents few cells. There is no pre-rectum. The rectum is inconspicuous and about as long as the anal body diameter. The continuous anus is also inconspicuous. The granules packed in the cells of the intestine are of variable size, the largest being about one-eighth as wide as the body; they do not give rise to a tessellated effect. The straight tail of the female is conoid to convex-conoid, and tapers from the anus, or from somewhat in front of the anus, to the sub-truncate or rounded, symmetrical terminus, which is two-thirds to three-fifths as wide as the base of the tail. There is no spinneret, and there are no caudal glands. There is a single lateral innervation on each side near the middle of the tail. The longitudinal fields are three-fifths as wide as the body. The excretory pore lies near the nerve-ring, which is oblique and of medium size. There is a posterior, rudimentary branch to the female sexual organs, about half as long

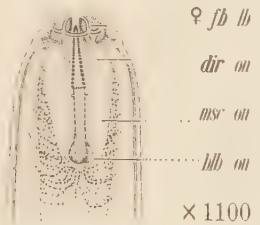

Fig. 1.—Lateral view of head of female of *Tylenchus mahogani, n. sp.* *fb lb,* framework of lip region; *dir on,* guide for spear; *msc on.* protruding-muscle of spear; *blb on,* bulbous base of spear.

as the body-width, and one-half as wide as long. From the well-developed elevated vulva, the large cutinized vagina extends inward at right angles to the ventral surface of the body. The uterus and ovary are outstretched toward the neck. The uterus is of such a size as to contain but one egg at a time. The uterine egg appears about twice as long as the body is wide and half as wide as long, and is covered by a thin, smooth shell. The eggs are deposited after segmentation begins; in fact, frequently contain embryoys. The medium-sized ovary is tapering in form, and near its blind end is only about one-third as wide as the body. It contains up to about twenty ova, which in the principal part of the ovary are arranged single file, but towards the blind end are arranged more or less irregularly.

Male: $\frac{3.2}{3.2}$ 13. $\frac{19.}{3.7}$ $\frac{4.}{4.4}$ $\frac{94.8}{2.8}$ 0.52 .. The two, equal, colorless, rather strong though slender, subarcuate spicula, at their widest part are one-fifth to one-sixth as wide as the corresponding portion of the body. They taper distally to a sub-acute apex. They are one and

one-half times as long as the anal body-diameter, are cephalated by a broad and shallow constriction, and are so arranged that their proximal ends appear to lie more or less dorsad from the body axis. The single, straight, or slightly arcuate, rather slender, more or less frail, simple, non-apophysate accessory piece lies parallel to the spicula and is about one-third as long as they. There are no supplementary organs. The rather plain-margined bursa springs from opposite the heads of the spicula at a distance in front of the anus as great as the corresponding body diameter. When seen in profile the somewhat

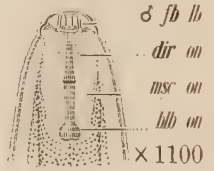

Fig. 2.—Lateral view of head of male of *Tylenchus mahogani, n. sp. fb lb,* framework of lip region; *dir on,* guide for spear; *msc on,* protruding-muscle of spear; *blb on,* bulbous base of spear.

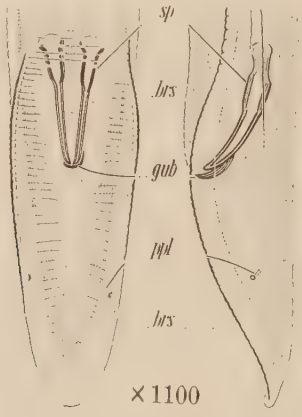

Fig. 3.—Ventral and lateral view of tail end of male of *Tylenchus mahogani, n. sp. sp,* spicula; *brs,* bursa; *gub,* gubernaculum or accessory piece; *ppl,* papilla.

rudimentary flaps of the bursa hardly reach the ventral contour. The bursa extends behind the anus a distance twice as great as the anal body diameter, just about encompassing the tail. On each side about midway on the tail, there is an inconspicuous lateral rib or papilla. There really is no very distinct flap to the bursa; so that in the ventral aspect the tail-end of the male appears widened only a very trifling amount, and the ventral surface only slightly concaved. Somewhat in front of the anus the two wings diverge, so that opposite the anus, they occupy two-thirds of the width of the body; here the dorsad

branch ceases, while the ventrad branch continues, and, expanding, forms the bursa, which is rather inconspicuous when seen laterally — rather less so than the illustrations would indicate.

Habitat: Tissues of the mahogany tree. Fixed in formalin, mounted and examined in water. This resembles *Tylenchus musicola* Cobb, but differs in the following respects:

1. It is relatively broader in the ratio of about 4 + : 3.
2. It is less coarsely striated.
3. The bursa is less obvious and not so thin.
4. The spear is more refractive, though relatively not so wide or long.
5. The guiding apparatus to the spear is more obvious.

The species also bears considerable resemblance to *Tylenchus coffeae* Zimmerman.

The following table give comparative data with reference to these species:

COMPARISONS

	Spear			Bursa
	Length and Width in Terms of Diameter of Base of Head		Nature and Composition of the Spear	
	Length	Width		
T. mahogani n. sp. ...	1	⅛	Refractive. Bulbs amalgamated	Not obvious, thick; not exceeding ventral contour; not exceeding tail
T. coffeae Zimm.	1-1⅓	¼-⅓	Refractive. Bulbs distinct	Obvious, thin; exceeds ventral contour; in length, equals tail
T. musicola Cobb.....	1⅓-1½	⅛	Faint. Bulbs distinct	Obvious, thin; exceeds ventral contour; not exceeding tail

It remains uncertain how serious this disease of the mahogany tree may be. The Director of Agriculture for Barbados, Mr. John R. Bovell, in a letter to the writer, says:

"I have known trees that have shown indications of being attacked for over thirty years which are still alive, and practically no different in appearance than they were when I first noticed them.

"I have been trying to see whether it was not possible to destroy the nemas by taking off the bark and the cambium layer practically down to the young wood of one-half of the attacked portion of the base of the tree and painting it with lime sulphur wash, repeating the painting in about a week and a half, allowing that half of the tree almost to heal before the other half was treated. The tree I treated bore the treatment exceedingly well and did not seem to feel the effects in the slightest, but I do not think we have succeeded in killing all the nemas."

CRITERIA FOR THE DIFFERENTIATION OF SCHISTOSOME LARVAE *

Ernest Carroll Faust

Stimulated by the practical phases of the Schistosome problem, several investigators have recently made notable contributions to the morphology and life-history of the Schistosome group. Cort (1919) has cleared up the details of structure of the cercaria of *Schistosoma japonicum,* while Leiper (1915) and Iturbe (1919) have demonstrated life-histories. Intensive study of this group of cercariae has made possible the ready separation of the human schistosome larvae from those of the group which infect other animals and, at the same time, has allowed a differentiation among the human species.

Thus the cercariae of the human schistosome species lack certain details of structure found in the non-human schistosome larvae. As far as the present knowledge indicates the former have no fluted margin to the tail trunk or furcae. They lack eye-spots. They have no pharyngeal sphincter around the esophagus. Moreover, they possess a smaller number of flame cells than is found in any other cercaria where the flame-cell formula is known.

The human schistosome cercariae have in common an integument covered in its entirety with heavy spines, usually somewhat heavier at the anterior end than near the furcal tips. They have a large pyriform oral sucker directed anteriad, but opening somewhat ventrad as Cort (1919) has shown. In the case of the cercariae of *Schistosoma haematobium* and *S. mansoni* the orifice is larger than in the cercariae of *S. japonicum* and is not conspicuously ventrad (see figures).

A conspicuous organ in the anterior region of the cercaria of the Japanese fluke is the head gland. I have found it to be decidedly basophilic in reaction, resembling the basophilic mucin glands of the larva of *S. mansoni.* Unlike this species, the other two species lack such an organ, by which means they may be readily separated from it. All three species have large conspicuous unicellular mucin glands with heavy ducts which open thru hollow boring spines anteriad and laterad with respect to the mouth.

Cort (1919: 500) describes these glands in detail for the cercaria of *Schistosoma japonicum.* There are five of them on each side with ducts passing ventrad to the nervous system and hence thru the heavy muscular region of the mouth. More recently he has discovered in

* Contributions from the Department of Pathology, Union Medical College, Peking, China. Read before the Section of General Medicine, China Medical Missionary Association Conference, Feb. 24, 1920.

his material what I have made out for the three known human species as well as for several non-human forms, that each duct is capped by a hollow boring spine, so that digestion of the host tissues and boring into them are performed by the same organ (Faust, 1919). These glands in the cercaria of *S. japonicum,* according to both Cort's and my observations on living material, are large organs with granular acidophilic cytoplasm and large nuclei with basophilic reaction. Thus with hematoxylin-erythrosin technic the cytoplasm stains a decided pink, while the nuclei take on a deep alkaline stain.

While probable differences in size and shape obtain in the case of the cercariae of *Schistosoma haematobium* and *S. mansoni,* which allow of their differentiation from the cercaria of *S. japonicum,* the number and type of the mucin glands is the most dependable basis of diagnosis. Thus the larva of *S. haematobium* has only three pairs of glands with a corresponding number of ducts opening strictly laterad to the orifice. The glands have small nuclei and give a simple acidophilic reaction. On the other hand, the larva of *S. mansoni* has six pairs of glands, with an equal number of ducts which are arranged around the orifice dorsolaterally in the form of two compressed crescents. Moreover, this species has the glands differentiated into two types. Two of the glands are acidophilic with large nuclei, while four give a basophilic reaction and have small nuclei. The basophilic glands take on a deep reddish hue with Best's calcium-ammonium-carmin stain. That the content of the gland undergoes a change as it passes into the duct is apparent from the fact that the granules around the nuclei of these basophilic cells are glycogeniferous, while the content of the duct gives a pure mucin reaction.

This method of distinguishing between these species of larvae makes it possible to diagnose two species in material which Dr. F. G. Cawston has sent the writer from Natal, namely, cercariae of *Schistosoma haematobium* and those of *S. mansoni.* The latter species corresponds both by structural and microchemical tests to Iturbe's species for Venezuela. This discovery of the larva of Manson's fluke in Natal is not entirely unexpected after Porter's discovery (1918: 45) of the adult fluke in South Africa. It shows further that the symptoms of rectal and urinary bilharziasis have not in themselves been sufficiently appreciated to discriminate between these parasites. Thus ability to recognize the larvae where a double infection obtains in a certain area adds a valuable check to the diagnosis and the combating of the disease.

A study of the immature larva of *S. mansoni* provided evidence of the fundamental difference between the two varieties of mucin glands in this species. At a stage before the furcae of the tail become

evident (Fig. 6) the two groups have already assumed their relative positions, have divided into the number characteristic of the adult and give the differential staining test.

It seems highly probable that the methods of specific diagnosis found valuable in separating these larval species may be used to equal advantage in other related groups.

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Explanation of Plate XV

Fig. 1.—Ventral view of the cercaria of *Schistosoma haematobium*, showing oral opening, mucin glands and ducts, and germ cells.

Fig. 2.—Lateral view of head of the cercaria of *S. mansoni*, showing relation of mucin duct openings to mouth.

Fig. 3.—Lateral view of head of the cercaria of *S. haematobium*, showing relation of mucin duct openings to mouth.

Fig. 4.—Lateral view of head of the cercaria of *S. japonicum*, showing relation of mucin duct openings to mouth.

Fig. 5.—Ventral view of the cercaria of *S. mansoni*, showing oral opening, mucin glands and ducts, and germ cells.

Fig. 6.—Ventral view of immature cercaria of *S. mansoni*, showing development of mucin glands.

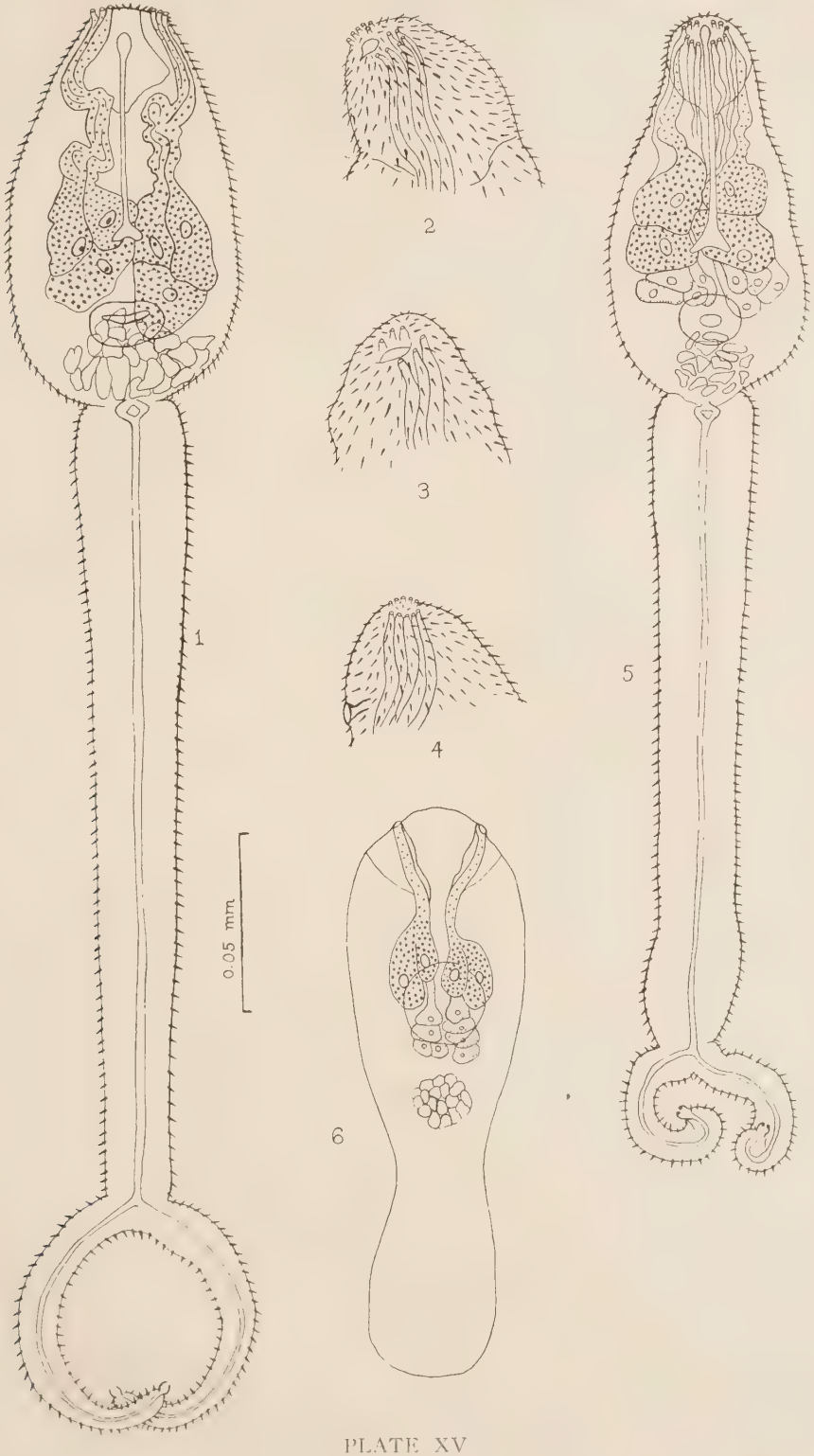
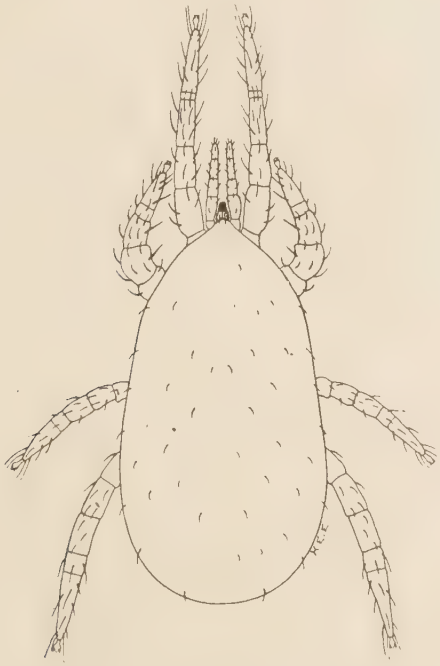


PLATE XV

A GAMASID MITE ANNOYING TO MAN

H. E. Ewing
Bureau of Entomology, U. S. Department Agriculture, Washington, D. C.

On two occasions during the summer of 1919 the writer has encountered a small gamasid mite that gets on the skin of the legs, and for that matter on any part of the body, and causes a considerable annoyance and a "creepy" sensation by running about over the skin. In addition to the annoyance thus occasioned, the mites have a faculty of stopping in the folds of the skin and inserting their mandibles, thus



Hyletastes missouriensis Ewing. Dorsal view, × 110.

causing actual pain. At present it has not been determined whether they engorge blood from man or not. On both occasions these mites were reported as chiggers by other people frequenting the same localities. One of these was along an electric line about five miles west of Washington, D. C., in Virginia, and the other was a front lawn of a friend about two miles from this place. The mite in question was described by the writer in 1909, from material sent to him by Professor

C. R. Crosby, from Columbia, Missouri. It was named *Hyletastes missouriensis*. The material in which the original specimens were contained consisted chiefly of bits of decaying leaves and had been obtained by using a Berlese trap. The mite has also been taken from under bark at Muncie, Illinois. A description of the species is here given:

A small, elongate mite of a uniform, light yellowish, brown color. Sexes alike as far as secondary characters are concerned. Body about twice as long as broad, sides subparallel behind the shoulders, and slightly concave in front of them. The abdomen is broadly and evenly rounded behind. Body all but naked above, yet observed to be very sparcely clothed with small simple setae, a pair of which is situated at the front apex. Ventral abdominal plate circular, slightly over one-half the width of abdomen in diameter; anal plate triangular, one-half as broad as ventral plate. Palpi about half as long as anterior legs and well clothed with setae. Mandibles stout; upper jaw or chela, a stout, projecting, strongly-curved, claw-like hook which surpasses the lower jaw, a short, sharp, curved sword-like, piercing structure; teeth not pronounced, and apparently confined to upper chela. Legs moderate, with rather small claws and ambulacrum. Anterior pair about as long as the body; tarsi about one and a half times as long as tibiae. Posterior legs extending for about one-third their length beyond the tip of the abdomen; tarsi over one and a half times as long as tibiae and divided near their bases. Length, 0.5 mm.; width 0.3 mm.

The potentialities of this species as a pest of man can not be predicted at present because of our lack of knowledge as to its biology and distribution. When it attacks in great numbers it is very irritating. No wheals or discolored spots are produced, hence it is easy to differentiate an attack of these mites from those of chiggers.

ALBERT FRANCIS COUTANT

Albert Francis Coutant was born in Brooklyn, New York, July 7, 1892, and died in Manila, P. I., April 18, 1919. He is survived by his wife, Mary Wotherspoon Stewart, of the Department of Botany of Barnard College.

Dr. Coutant received his B.S. degree from Cornell University in 1913, and his Masters degree in 1914. In 1917 he received his degree of M.D. from the same institution. He was student assistant in Entomology at Cornell from 1911 to 1914, and in the summer of 1912 was assistant in Zoology at the University of Illinois. As an undergraduate his special work in Entomology was largely from the viewpoint of parasitology, and this soon broadened into an interest in the general field.

During the summer of 1916 he worked under the International Health Board of the Rockefeller Foundation on the eradication of the hookworm in Texas. After graduation from the medical college he became established in the Cancer Memorial Hospital in New York, but in September, 1917, accepted an appointment tendered jointly by the International Health Board and the Philippine Health Service to become Chief Surgeon on the Hospital Ship *Busuanga,* operating among the Moros in the southern end of the Philippine Archipelago. During the first six months of 1918 while the ship was undergoing repairs, Dr. Coutant was acting superintendent of St. Luke's Hospital, one of the largest in Manila.

Though devoted to his work, he wrote shortly before his death, "The desire to go back to teaching is still strong with me." The writer happens to know of two tempting university positions in parasitological work which were offered to Dr. Coutant, but he felt that he was under obligations to continue on the hospital ship until the completion of his three year term. This attitude of faithfulness and loyalty was typical of all of his relations in life.

Though his publications were few, he was a keen observer, and accumulated many data which he was planning to utilize in future work. At the time of his death he had several papers in course of preparation, but unfortunately they were not in shape to be completed by another.

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THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

Thirtieth to Thirty-Eighth Meetings, 1916-1919

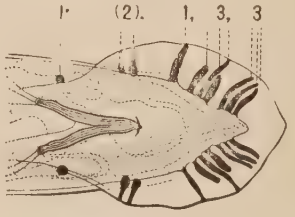
The thirtieth to the thirty-seventh meetings were held at intervals during the years 1916 to 1918. The following includes a few of the papers and notes presented, most of those not reported having been already published or seeming to be no longer of special parasitological interest.

At the thirtieth meeting, March 3, 1916, Doctor Cobb was elected president, and Mr. Crawley secretary.

At the thirty-second meeting, May 12, 1916, Doctor Cobb presented the following note:

BURSAL FORMULA FOR RHABDITIS

As the bursa in this genus is symmetrical, only the papillae and ribs on one side of the bursa are considered, and these are represented by rather arbitrary designations grouped in a formula. These organs are designated according to their proximity to each other and not according to their anatomical and physiological characters. The papillae and ribs are considered as a single longitudinal series, and each group in the series is indicated by a digit representing the number of ribs or papillae in the group. They are regarded as either anal, pre-anal, or post-anal, according as they are opposite to, in front of, or behind the anus. In the formula the anus is included in the parentheses; all papillae approximately opposite the anus are included in the parentheses, the pre-anal papillae are placed in front, and the post-anal papillae after the parentheses. The longitudinal spaces or distances separating the groups of papillae and ribs are



Tail end of a male *Rhabditis*, showing spicula, anal opening, bursa and ribs of the bursa. The ribs of the bursa are shown black. The grouping of the ribs is indicated by the figures above; the corresponding formula as it is to be printed, is shown below the bursa.

indicated by commas and semicolons, the comma representing a short distance, the semicolon a long distance. In some cases before and after the parentheses the punctuation mark may be omitted, thus indicating that the ribs or papillae are even nearer to the anus than in those cases where the separation is indicated by a comma or semicolon. A blank space in the type after the comma, or after the semicolon, indicates a longer distance than is indicated by the comma alone or by the semicolon alone. By these simple means it is easily possible with ordinary type to indicate in a compact formula with considerable accuracy the grouping and latitude of these various elements of the bursa. A glance at the adjacent illustration and formula will make the matter quite clear.

At the thirty-fourth meeting, December 21, 1916, Doctor Stiles gave an account of an investigation made by him and Doctor A. D. Weakley, having for its object to ascertain what connection *Endamoeba gingivalis* has with pyorrhoea alveolaris or Riggs' disease. The study was made on the inmates of the Government Hospital for the Insane, Washington, D. C.

Tests were made with emetin, injected hypodermically daily for six days. In 27 cases, all having endamoeba, the drug was given from July 24 to 31. On

September 22 examinations were made in 25 of these cases with the following results: Marked improvement, 3 cases; slight improvement, 8 cases; no improvement, 14 cases.

The above refers to the conditions as found in the mouth. With regard to the effects on the parasite, 12 showed it 4 days after treatment; 6 showed it 10 days after treatment; 4 showed it 31 days after treatment; 2 showed it 59 days after treatment. In one typical case of Riggs' disease, the amoeba was not present.

The conclusions are to the effect that Riggs' disease is not due to the amoeba and that the amoeba is not always destroyed by emetin.

At the thirty-fifth meeting, January 19, 1917, following a discussion by Doctor Hadwen of investigations on reactions of animals to parenteral injections of juices secured by crushing the bodies of parasites, the results of which have been published elsewhere, Doctor Ransom presented the following note:

REACTIONS FOLLOWING INJECTION OF PARASITE MATERIAL

Experiments have been carried out on the effects of injecting into animals material obtained from various species of metazoan parasites, such as body fluids and aqueous extracts or suspensions of their tissues, either fresh or dried and pulverized. These experiments were suggested by the recent work of Hadwen, of the Canadian Department of Agriculture. In the experiments the host animals used were cattle, horses, sheep, hogs, dogs, cats, rabbits, rats, guinea-pigs, turkeys and chickens, and the parasites included nematodes and tapeworms of various species, ticks, lice, warbles and bots. Few experiments were made on the ophthalmic and intradermal reactions, and the injections in most cases were given subcutaneously, occasionally intravenously. The conclusions reached in some respects are slightly different from those first expressed by Hadwen. Some of the more important are as follows:

Reactions of an anaphylactic type may be produced in cattle, sheep and hogs by single injections of antigens prepared from various metazoan animal parasites.

In some cases the reaction may possibly be specific and dependent upon the existence of infection with the species of parasite from which the antigen is obtained.

In other cases there is no relation between the reaction and the presence or absence of parasites of the species from which the antigen is obtained, and animals may react to parasites of species with which they are not liable to infestation.

Sheep are very susceptible to injections of crushed material, fluids, or extracts from certain metazoan parasites, irrespective of the presence or infestation with these parasites, and small quantities, which have no apparent effect upon guinea-pigs and rabbits when injected intraperitoneally or subcutaneously, when injected subcutaneously into sheep produce severe reactions, frequently terminating in death.

Sheep may respond repeatedly to subcutaneous injections, at intervals of a few days, of material from the same species of parasite, so that the reaction in sheep apparently differs from the ordinary anaphylactic reaction not only in the fact that a sensitizing injection is not required, but in that sheep recovering from one reaction are not thereafter for a considerable period of time insusceptible to further injections.

It is believed that investigations in the field opened up by Hadwen's work will be found to have an important bearing upon the many problems relating to the phenomena of anaphylaxis, and as Hadwen's reaction (that is, the response of animals to antigens prepared from metazoan parasites) in some cases appears to be specific, it may prove of practical utility in diagnosis.

At the thirty-sixth meeting, October 26, 1917, Doctor Ransom was elected president, Mr. Crawley continuing in office as secretary.

Doctor Stiles presented the following note:

A SECOND CASE OF GONGYLONEMA IN MAN

Birge of the Florida State Board of Health has recently seen a case of Gongylonema in a white girl. The case is similar to that recently reported by H. B. Ward. The worm may be either *G. pulchrum* or *G. scutatum*.

The thirty-eighth meeting was held at the residence of Doctor Hall, October 18, 1919. Doctor Ransom was reelected president and Doctor Hall was elected secretary.

Doctor Cobb presented the following note accompanied by a demonstration of the doubly refractive cell inclusions in the intestinal cells of a nematode:

THE USE OF THE POLARISCOPE IN DETERMINING THE CHARACTER OF CELL INCLUSIONS IN NEMAS

In a former paper (J. Parasitol., 1: 40-41) read before this society, attention was called to the presence of doubly refractive bodies in the intestinal cells of *Rhabditis monhystera* Bütschli, the name rhabditin being given to the material of which these bodies are composed. In connection with the importation of plants and soil in order to exclude harmful species of nemas that are likely to be present in the small quantities of soil sometimes adhering to the roots of imported plants or in soil brought in as ship ballast, it is important that some broad lines of differentiation be found between harmful and harmless or beneficial nemas, particularly since the imported nemas are commonly of unknown species with unknown food habits. With reference to such a distinction, the granules of the intestinal cells are of interest. As, broadly speaking, the granules are related to the character of the food, the nemas of the two large groups may be expected to show granules of two large general groups. Fortunately, in some nemas food habits are well known. An examination of the intestinal granules of herbivorous nemas and of the less common carnivorous nemas indicates that carnivorous forms that show birefringent granules are approximately twice as numerous as those that do not, whereas the reverse is true for herbivorous forms. Aside from calcium sulphate and rhabditin, five or six kinds of doubly refractive granules have been found in the course of an examination of almost two hundred species of nemas, belonging to about forty genera, and these granules fall into two groups. One of these groups comprises granules that are evidently stored food material, and the other granules that are evidently elimination material; one is anabolic and the other katabolic. The granules of the first group are abundant when present, sometimes comprising more than 25 per cent. of the cell volume. Further study will be made in the hope of distinguishing between herbivorous and carnivorous nemas on the basis of the granules.

Doctor Cobb also presented a note on an adaptation of the polariscope to immersion lenses. In this adaptation the nickel prism is mounted very close to the back lens of the objective. The condenser of the microscope is replaced by an immersion lens, and the object to be examined is mounted between two cover glasses. This apparatus is of great value in studying the cell inclusions in nemas, many of which are on the limits of visibility.

Doctor Ransom presented the following note:

GAPEWORM IN TURKEYS AND CHICKENS

Investigations have shown that the gapeworm of poultry (*Syngamus trachealis*) is found commonly parasitic in turkeys. Feeding experiments show that young chickens readily become infected, but that older birds are comparatively immune, and as a rule cannot be infected by feeding material which is infectious for chicks. At least the worms rarely develop to the mature stage in adult chickens, and when they do succeed in reaching maturity they often

appear to remain in the trachea but a short time, and the chickens soon become free from infestation. On the other hand, adult turkeys can be easily infected as well as young poults, and apparently they can harbor the parasites during long periods. The results of experiments on chickens and turkeys have been confirmed by postmortem observations on birds slaughtered for market purposes. Adult chickens are habitually found free from gapeworm, as shown by an examination of 635 chickens from Center Market, Washington, D. C., all of which were negative. Adult turkeys, however, are commonly found infested. Out of 679 turkeys examined at Center Market, 153, or 22.5 per cent., were infested with gapeworm.

From the foregoing it appears that adult chickens are comparatively of little importance as gapeworm carriers. Adult turkeys, on the contrary, are of major importance as carriers of gapeworms, although they are not likely to be suspected by the poultry raiser as spreaders of infection, since they commonly show no outward symptoms of disease. Turkeys, therefore, must be given consideration as reservoirs of infection as well as the soil in which, according to the results of experiments upon the longevity of gapeworm larvae, infection may persist under favorable conditions for over a year.

Young gapeworms may be found in the lungs within a week after feeding infective material, and the two sexes become coupled in the lungs while still very small. Later they migrate to the trachea, and oviposition begins within two weeks after the feeding of infective material. Gapeworm larvae in guinea-pigs will migrate to the lungs and undergo an incomplete development.

MAURICE C. HALL, Secretary.

BOOK REVIEWS

THE AMOEBAE LIVING IN MAN. A Zoological Monograph. By Clifford Dobell, M.A., F.R.S. New York, William Wood & Company, 1919.

The scientific world is deeply indebted to Doctor Dobell for his recent monograph on the human amoebae. His work, which is truly monographic in character, covers a field that is in a state of serious confusion. To one not intimately familiar with the organisms and unable for any reason whatever to devote much time to the study of previous publications, it is impossible to reach clear conclusions regarding the many questions in dispute. Doctor Dobell has that thorough knowledge which lays the sure foundation for such a study. His long series of valuable studies represented by shorter publications of recent years, the responsibility of training the English workers who devoted themselves to the subject of amoebic dysentery during the war, and membership on the War Office Dysentery Committee made him thoroly familiar with the work done under English auspices. A tireless laboratory worker as well as a keen and impartial critic of the literature, no one else could be named who is anything like as well fitted to give an impartial view of these controversial questions.

Those in any field who are interested in the amoebic parasites of man will find in the volume a work of great interest and helpfulness. After a brief introduction and a useful section on materials and methods, Doctor Dobell reviews concisely the present state of knowledge concerning human amoebae, and then discusses the genera, closing with the following synopsis to indicate the acceptable names and the synonyms:

SYNOPSIS OF GENERA AND SPECIES OF AMOEBAE LIVING IN MAN

Genus I.—*Entamoeba* Casagrandi and Barbagallo, 1895 (nec *Endamoeba* Leidy, 1879).
Synonyms:
Poneramoeba Lühe, 1908.
Löschia } Chatton and Lalung-Bonnaire, 1912.
Viereckia }
Proctamoeba Alexeieff, 1912.
[*Amoeba* (*pro parte*), *Endamoeba*, *Entamoeba*, *Endameba*, *Entamöba*, Auctt.]
Type: *E. coli* (Grassi) Casagrandi and Barbagallo.
Species in Man: *E. coli* (Grassi) Casagrandi and Barbagallo.
E. histolytica Schaudinn (*emend, Walker*).
E. gingivalis (Gros) Brumpt.

Genus II.—*Endolimax* Kuenen and Swellengrebel, 1917.
Only species, hence type: *E. nana* (Wenyon and O'Connor) Brug.

Genus III.—*Iodamoeba* nov. gen.
Only species, hence type: *I. bütschlii* (Prowazek) Dobell.

Genus IV.—*Dientamoeba* Jepps and Dobell, 1918.
Only species, hence type: *D. fragilis* Jepps and Dobell.

In subsequent chapters each of these genera and species is studied in detail and a complete analysis given of the structure, life history, clinical relations and nomenclature. It would be impossible to review here the immense amount of detailed information compressed into the closely printed pages of the monograph. This section may be commended to the careful study of parasitologists and of clinicians who desire to know the correct form of the names for the various species and the basis on which these conclusions are reached.

Dobell protests rightly against the suppression of the diphthong in the name Amoeba, which has to some extent crept into our literature, probably through the adoption of a quasi-common name ameba. There is certainly no justification for attempting to depart from the Latin language and to modify the original spelling. Dobell also makes it very clear that the genus designation of the human parasite is properly Entamoeba and that the genus Endamoeba, originally

described by Leidy in 1879 for a species of amoebae found in the cockroach, must be preserved for that type.

Some American workers may not look kindly upon the use of the form *Entamoeba histolytica* of Schaudinn in preference to *E. dysenteriae* of Councilman and Lafleur, which has come into use in some circles, but Dobell's argument is unanswerable, and as shown by Stiles some years ago, the name *E. dysenteriae* cannot be justified.

The monograph contains also sections on the amoebae in human urine, in dogs, and in monkeys, as well as on certain other amoeboid organisms described from man which are not true parasitic amoebae of man, but are to be explained in one way or another. They are but a small section of the long list of pseudo-amoebae that could be compiled from the literature of parasitology and medicine.

The work closes with a good bibliography, following which are five plates illustrating the forms under discussion. The colored plates are especially worthy of mention, since they represent in a particularly faithful manner the appearances that present themselves under the microscope to those working with stained and mounted preparations.

The Fifth and Sixth Reports of the Director of Veterinary Research in the Union of South Africa make a splendid volume of scientific contributions in which are some papers of marked interest to parasitologists. Special mention might be made of the work on intoxication by Gastrophilus larvae. It is due to a toxin, but in the view of the authors the symptoms do not accord with anaphylaxis. The life history of a new nematode from fowls (*Filaria gallinarum*) shows developmental stages in termites on which the fowls feed habitually.

NEW HUMAN PARASITES

Monas urinaria Reitler and Robicsek, 1920. — This species of flagellate was observed in the urine in four cases, two of cystitis, one of nephritis, and one of tuberculosis at an army hospital in Vienna. Biflagellate, ameboid, cystic and multinucleate monoflagellate stages are described. The organism was found in the urine only after standing a few hours, and could not be discovered in prostatic or urethral secretions, nor in urine obtained in a sterile condition. The authors therefore conclude that the organism is a free-living form and not a human parasite or commensal (Cent. Bakt., I. Orig., 84:129-132, 1 pl.; Feb. 11, 1920).

Trypanosoma escomeli Yorke, 1920.—Escomel (1919; Bull. Soc. path. exot., 21: 723) described a case of trypanosomiasis from the tropical forests in the eastern portion of Peru. He identified the parasite as probably *Schizotrypanum cruzi*, but Yorke considers that it is probably not of this species because of its larger size (up to 40μ) and because of its small, hardly visible blepharoplast. Accordingly, Yorke proposes the name given above for Escomel's trypanosome. (Ann. Trop. Med. and Parasitol., 13: 459-460; March 15, 1920.)

Spirochaeta orthodonta Hoffmann, 1920. *Spirochaeta skoliodonta* Hoffmann, 1920. *Spirochaeta trimerodonta* Hoffmann, 1920.—In an article in which he pays little attention to the rules and customs of zoological nomenclature, Hoffmann discusses various forms of spirochetes that occur in the human mouth, including *Spirochaeta buccalis crassa, S. buccalis tenuis, S. media oris,* and the three others named above. *S. skoliodonta* and *S. trimerodonta* are proposed as new species. *S. orthodonta* is a name proposed as a substitute, apparently in order to secure a sort of uniformity in names, for a species formerly known as *Spirochaeta dentium* or *S. denticola* (Deutsche med. Wchnschr., 46:257-259, 1 fig., March 4, 1920).

Diplocercomonas soudanensis—Because the original generic name was preoccupied, the form noted previously as *Dicercomonas soudanensis* (Jour. Par., 6:48) has been renamed as above by the authors (J. Trop. Med. & Hyg., 22:190; Oct. 15, 1919).

NOTE

Research in the field of Parasitology has suffered a serious loss in the sudden death from infective jaundice of Doctor A. J. Chalmers (*aet.* 50) at Calcutta on April 6. When Doctor Andrew Balfour left Khartoum, Doctor Chalmers became his successor there as Director of the Wellcome Tropical Research Laboratories which already enjoyed a world wide reputation. Doctor Chalmers maintained this reputation and extended it. He had resigned his post as Director and was in India on his way home when stricken down.

Doctor Chalmer's own work was of the highest type, abundant in quality and characterized by both accuracy and thoroughness. It was also marked by the generous appreciation accorded the work of colleagues and the full measure of credit given to associates in the laboratory. In addition to numerous separate publications in parasitology, especially on mycetoma, Doctor Chalmers is most widely known as author of the splendid Manual of Tropical Medicine written in cooperation with Doctor Aldo Castellani.

INDEX TO VOLUME VI

The actual dates of issue of Volume VI of THE JOURNAL were as follows:

No. 1, October 29, 1919
No. 2, January 24, 1920
No. 3, May 19, 1920
No. 4, Aug. 14, 1920